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(*Recognosce notum, ignotum inspice*)

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NOTES ON ENTOMOGENOUS FUNGI

By T. PETCH

(With 7 Text-figures)

76. *Cordyceps Engleriana* P. Henn.

Cordyceps Engleriana was described by Hennings in 1897 (*Engl. Bot. Jahrb.* XXIII, 539) from a specimen on a spider from the Cameroons. From the host arose numerous slender clavae, up to 15 mm. long, some of them conidial and others perithecial. The conidial clavae were branched above, blackish brown, and bore oblong basidia, $6–7 \times 3{\cdot}5–4\mu$, with ovoid-ellipsoid, hyaline conidia, $4–5 \times 3–3{\cdot}5\mu$. The perithecial clavae were blackish below and yellowish ashy above. The perithecia were situated in a cluster on one side of the clava at the apex, and were superficial, cylindric, fusiform, with an obtuse, rounded apex, 1–1·5 mm. high and 0·25 mm. diameter. Hennings stated that the perithecia looked like small insect pupae.

The foregoing details are taken from Hennings's description. The type is now preserved in alcohol as an exhibition specimen. The specimen conforms to Hennings's description and figure, but further details of the conidial stage cannot be obtained. The perithecia are superficial, elongated oval or cylindric.

From Mr A. W. Bartlett, I have received a British Guiana specimen which appears to be this species. The host is a large spider, seated on the leaf of a Monocotyledon. From the body and legs of the spider, there arise twenty-one clavae, scattered or sometimes two together. The clavae are up to 15 mm. high, linear, variously twisted and curved, sometimes forked above, blackish brown or black. Some clavae are conidial, and bear cylindric basidia, 18μ high, 3μ diameter, continuous or one-septate, rounded and thick-walled at the apex, with a minute, central sterigma. This is evidently a *Hymenostilbe*, but conidia were not found. The perithecial clavae are slightly thickened towards the apex, and there bear the perithecia along one side, usually clustered, with a few outlying scattered. The perithecia are 0·6 mm. high, 0·3 mm. diameter, superficial, elongated ovate or slightly flask-shaped, apex obtuse. The asci are 5μ diameter (not $8–10\mu$, as given by Hennings), and the fungus is a *Cordyceps*, not *Ophiocordyceps*. The specimen is an old one, and the perithecia are now brown, the wall being red-brown by transmitted light.

The spider is attached to the leaf by a fringe of tawny brown mycelium

along its legs. In the type specimen, the spider appears to be merely clasping a stem, without any mycelial attachment, as far as can be determined by inspection through the glass of a museum jar.

In 1901, Möller (*Phycomyceten und Ascomyceten*, p. 208, fig. 97) figured and briefly described *Cordyceps flavo-viridis* from Brazil. This species was found on the leaf of a *Calathea*, on which it formed a closely adpressed, fleecy, loose stroma, arising from some small brown pupae. From the stroma, densely interwoven, greyish white strands, about 1 mm. broad, extended over the leaf in various directions, and on these strands were situated superficial, elongated flask-shaped, yellow-green perithecia, 0·5 mm. high, singly or in groups. Möller believed that the fungus was parasitic on the brown pupae, two of which are shown in his figure.

Inspection of the type specimen, through the wall of a museum jar, causes some doubt of Möller's interpretation. The repent strands appear to arise from a mass of debris, which is apparently situated beneath a thin silk sheet, such as is constructed by a spider on the under surface of a leaf. When a spider beneath one of these tents is killed by a fungus, the fungus grows out, sometimes through the silk sheet, but more usually along its margin, which is not attached to the leaf all round. Moreover, other insects may take refuge underneath the sheet, and a dead spider is often devoured by scavengers. In the type specimen of *Cordyceps flavo-viridis*, the minute pupae appear to be quite clean and not attacked by the fungus. It would seem probable that these are the pupae of some intrusive insect, perhaps a Dipteron, and have no relation to the fungus.

Möller remarked that *C. flavo-viridis* was a *Torrubiella*, according to the definition of the latter genus, but if the repent strands which bear the perithecia were only a little more firmly compacted, so that they could stand erect, the difference between *Cordyceps* and *Torrubiella* would disappear.

In that case, however, the fungus would be *Cordyceps Engleriana*, with which its large free perithecia agree. I suggest that *C. flavo-viridis* is a small example of *C. Engleriana*, and that it is parasitic on a spider, not on the pupae which occur in the mass of mycelium. That can be decided only by examination and dissection of the type specimen.

In *Mycological Notes*, No. 69 (July, 1923), p. 1197, C. G. Lloyd wrote, "*Isaria flavo-viridis* from Rev. J. Rick, Brazil (fig. 2445). As named by Rev. Rick, or rather as *Cordyceps*, but I would rather record it as *Isaria* until its *Cordyceps* nature is proved. The name is quite appropriate to express its color. Growing on a pupa it no doubt is a *Cordyceps* or will develop into one. I do not like to cut the only specimen, but on scraping it I find only greenish hyphae. No sign of perithecia or even conidial spores." The figure shows an erect

cylindrical clava growing from a pupa and has no resemblance to Möller's illustration. This is probably a case of the inadvertent use of a prior name.

77. *Cordyceps ainictos* Möller

In *Phycomyceten und Ascomyceten* (1901), Möller described, under the name *Cordyceps ainictos*, a peculiar specimen which appeared to have two different kinds of perithecial clavae arising from the same insect. In the one kind, the perithecia were totally immersed in a lateral pulvinate head; in the other, they were completely superficial on long linear clavae. As Möller pointed out, these represent the two extremes of perithecial arrangement in the genus *Cordyceps*. The superficial perithecia on the long linear clavae were all effete, and consequently Möller had to leave the identity of the two forms in doubt, though he inclined to the belief that they were two different species growing from the same larva.

Through the kindness of Dr R. Heim, I have recently been able to examine a specimen from Madagascar, which resembles exactly Möller's figure, except that the long clavae are stouter. It grew on a buried larva, but it was not possible to determine what kind.

In the first form, the perithecial clavae are up to 1·5 cm. high. The stalk is about 0·5 mm. diameter below, attenuated regularly to the apex, terete, sometimes flattened and expanded at the base, red-brown, pruinose. The head develops half-way up the stalk, at first lateral and pulvinate or subglobose, becoming irregularly lobed and encircling the stalk; it is up to 2 mm. diameter, pale brown, dotted with rather large, dark brown, subtranslucent ostiola, which do not project. Thus, the position of the head is similar to that in *Cordyceps unilateralis*, but the colour and texture of the fungus are quite different and resemble those of *C. dipterigena*. The head has a definite cortex, and the perithecia are immersed, perpendicular to the surface, in a peripheral layer. The perithecia are narrow flask-shaped, 0·6 mm. high, 0·25 mm. diameter, the perithecial wall being pale amber, stout, about 25 μ thick. The asci are narrow cylindric, capitate, 4–5 μ diameter, with linear ascospores in a parallel bundle. The free part-spores are cylindric, becoming slightly narrow oval, 2·5–9 × 1 μ.

In the second form, the linear clavae are up to 2·5 cm. long, 1 mm. broad, flattened (strap-shaped), twisted, longitudinally furrowed, dark brown, glabrous, sometimes giving off brown, ashy pruinose, terete branches, perpendicular to the main clava, up to 1 cm. long, 0·25 mm. diameter. Towards the distal end, the long clavae are covered with scattered or crowded, superficial perithecia, up to 0·4 mm. high, 0·25 mm. diameter, conico-cylindric or ovate, pale brown, pruinose, thin-walled, apex obtuse. Unfortunately, all the perithecia examined were either effete or immature.

On Möller's specimen, the lateral branches of the linear clavae (the second form) bear, in some instances, perithecial heads of the first form. In the Madagascar specimen, the lateral branches of the linear clavae are conidial, the conidial stage being a *Hirsutella*. The *Hirsutella* phialides are flask-shaped, 9–11μ high, 2μ diameter below, attenuated into a stout sterigma. The conidial cluster is limoniform, $3 \times 1{\cdot}5\mu$, and the individual conidia are cymbiform, $2{\cdot}5 \times 0{\cdot}75\mu$. These dimensions are smaller than is usual in *Hirsutella*, and it is probable that they may be abnormal.

Although it is not possible to arrive at a complete solution of this puzzle, it would appear most probable that the superficial perithecia are those of a *Byssostilbe* or other Nectriaceae, parasitic on the *Cordyceps*. In that case, the whole of the specimen, except the superficial perithecia, belongs to one species, the linear clavae being conidial clavae of the *Cordyceps*.

Theissen (*Ann. Myc.* IX (1911), 68) took the long linear clavae to be *Cordyceps ainictos* Möller, and under that name gave a description of a specimen with linear clavae and superficial perithecia, which he had found in Brazil. But the only identifiable *Cordyceps* which Möller had was the form with pulvinate lateral heads, and it is clear that that form must take Möller's name. Theissen's specimen was not the same species.

Cordyceps corallomyces Möller is probably the same as *C. ainictos*. Möller figured a head with strongly projecting perithecia, but he stated that the latter were not superficial, but covered by a definite continuous cortex. In the type specimen, the lateral heads resemble those of *C. dipterigena*, and it is probable that the one figured by Möller is an extreme variation, parallel to that which occurs in the latter species (*Trans. Brit. Mycol. Soc.* X (1924), Pl. I, fig. 6). Against that supposition is the fact that Möller described *C. corallomyces* as red, and it is bright red in his coloured figure in the Berlin Herbarium.

78. *Cordyceps Lunti* Giard

In Saccardo, *Sylloge Fungorum*, XIV (1899), 662, *Cordyceps Lunti* Giard is included, with references to *Bull. Soc. Entom. France* (1895) and *Revue Mycologique* (1899), p. 11. The fungus was said to have occurred on an elaterid larva in France.

The second reference is to a translation, by Ferry, of Massee's *Revision of the genus Cordyceps* in *Ann. Bot.* IX (1895). Ferry added to Massee's paper an appendix which included species described by Quélet and Giard subsequent to its preparation. Among the latter is *Cordyceps Lunti* Giard, for which Ferry did not give any locality.

On referring to Giard's original description, however (*Bull. Soc. Entom. France*, LXIV (1895), p. clxxxi), it is found that the name is

Cordyceps Hunti, and that the fungus was from Trinidad. It had been collected by W. Hunt, and sent to Giard by J. H. Hart. Saccardo evidently took his description from Ferry's paper. Similarly, all references in the *Sylloge Fungorum* to Massee's paper are to Ferry's translation in the *Revue Mycologique* (1899), instead of to the *Annals of Botany* (1895), and even in the *Index Iconum*, vol. XIX, Massee's figures are cited from the copies in the *Revue Mycologique*.

Giard stated that the clavae and the mycelium which covered the larva were orange-red. Two clavae were present, one almost 5 cm. long, and the other, 3·5 cm. long, regularly thickened upwards, with a conical apex. The stalk was up to 2 cm. long and 1·5 mm. diameter, while the thickest part of the clava was 2·5 mm. diameter. The two clavae had coalesced for a length of 4–5 mm. The perithecia were irregularly scattered, more abundant on one side than on the other, and absent from a small area at the apex, which was not otherwise clearly defined from the remainder of the clava. Apparently the perithecia were immersed, as Giard stated that they formed minute brownish dots on the red clava, presumably referring to the ostiola. Asci and spores were not recorded.

The description agrees with *Cordyceps martialis* Speg. (*C. submilitaris* P. Henn.). *C. Hunti* should be regarded as another synonym of *C. martialis*.

Cordyceps Pittieri Bomm. & Rouss. (*Bull. Soc. Bot. Belge* (1896), p. 160) was described from a specimen on a beetle larva in wood from Costa Rica. The description would fit *C. martialis*, as far as it goes, but the colour could not be stated, as the specimen had been preserved for a long time in alcohol.

79. *Cordyceps Melolonthae* (Tul.) Sacc.

In 1824, Jacob Cist contributed a paper, "Notice of the Melolontha or May Bug," to *Silliman's American Journal of Science and Arts*, Ser. 1, VIII, 269. In it he mentioned the occurrence, on the larvae of the beetle, of a fungus which sometimes was three inches high. No mycological details were given, and all the information about it is embodied in the first paragraph of Cooke's account of *Cordyceps Melolonthae*. His specimens were North American.

Cist gave three figures. One of them shows two clavae, about one inch high, clavate above, arising from the larva. The other two show single, terete, cylindric clavae, three to four inches long, fimbriate at the apex. It is evident that, if these were *Cordyceps* clavae, they were quite immature.

In *Selecta Fungorum Carpologia*, the Tulasnes named Cist's fungus, *Torrubia Melolonthae*. They had no specimens, but based their name on Cist's figures.

Previously, in 1769, Fougeroux de Bondaroy had described similar fungi on cockchafer larvae from the West Indies. His figures show cylindric clavae which are, in some, slightly inflated at the apex, as though developing an ovoid head. Three of Fougeroux de Bondaroy's figures were reproduced by Cooke (*Vegetable Wasps*, etc., p. 97, text-figs. 20–2).

The Tulasnes referred Fougeroux de Bondaroy's figures to Cist's fungus, *Torrubia Melolonthae*. As, however, they had not examined specimens of either fungus, and both those figured would appear to have been immature, it is quite possible that they are different species. In that case, the name would belong to the North American species.

As no description of *Cordyceps Melolonthae* was possible, and Cist's figures do not represent a mature *Cordyceps*, the name was practically *nomen nudum*. But specimens which corresponded with Cist's figures were apparently frequently collected in North America. It was a common parasite on "White Grub," *i.e.* the larva of a cockchafer, but was not known in the mature state.

Meanwhile, a *Cordyceps* which was occasionally found on "White Grubs" in North America was referred to *C. herculea* (Schw.) Sacc. It was described and figured under that name by Hard in *Mushrooms, edible and otherwise* (1908), and by Seaver in *The Hypocreales of North America* (1912), the figures in the latter being drawn from a photograph. In Herb. Kew., there is a copy of a similar photograph of an American specimen from W. C. Sturgis.

Lloyd, however, in *Letter* 47, November, 1913, p. 16, note 98, stated that he had examined the type specimen of *Cordyceps herculea* (Schw.) Sacc., and found that it was *Cauloglossum transversarium*. He claimed that the "White Grub" fungus was *Cordyceps insignis* Cke. & Rav. Subsequently (*Mycol. Notes*, No. 39, December, 1915, p. 530, fig. 722), Lloyd adopted the name *Cordyceps Melolonthae*, with *C. insignis* as a synonym, for the American "White Grub" *Cordyceps*.

Hard's photograph of "*Cordyceps herculea*" shows a specimen about 6·5 cm. high, with a stout, cylindric stalk, 2·75 cm. high and 1 cm. diameter, and a broader cylindric head, 4 cm. high, 1·8 cm. diameter, rounded above with a small central knob. The perithecia are apparently immersed, and the lower edge of the head is somewhat irregular, but does not show a re-entrant V. Hard states, "The plant is quite large, clavate in form, the head oblong, round, slightly tapering upwards, with a decided protuberance at the apex. . . . The head is light yellow."

Seaver's figure (*op. cit.* Pl. 53, fig. 6) shows two clavae developing from one larva. The larger is 5·5 cm. high, with a stalk 8 mm. diameter, and the other, 4·5 cm. high, with a stalk 5 mm. diameter. In both, the fertile part of the clava consists of two longitudinally oval, thickened areas, one on each side of the clava, partly in contact.

Presumably these areas would have extended over the clava until they fused into a continuous head. Seaver described the fungus as "head enlarged and more than 1 cm. thick, with the fertile portion often interrupted, leaving bare patches and in the specimens examined terminated by a short obtuse apex; fertile portion roughened by the slightly prominent necks of the perithecia." From the figures, the obtuse apex is sterile.

Fig. 1. *Cordyceps Melolonthae*, from Santa Fe de Bogota. $\times 1\frac{1}{8}$.

Sturgis's photograph is similar to Seaver's figure, but the lateral fertile areas are less developed. Sturgis stated that the colour was livid yellow, and that the part-spores measured $12{\cdot}8 \times 2{\cdot}2\,\mu$.

In a specimen in Herb. British Museum, from "Santa Fe de Bogota," the head is developing from three longitudinal areas, which have not completely fused.

In specimens in Herb. Kew., from South America, several clavae arise close together near the head of the larva. These are all small, the largest being 2·4 cm. high, with a cylindrical head, 14 mm. long, 3 mm. diameter. The whole of the head is fertile, except for a V-shaped groove at the base on each side. One of these specimens, *ex* Herb. Currey, is marked "*Cordyceps* on a larva which destroys the cotton crop in S. America," and was referred by Massee to *Cordyceps sobolifera*.

The most peculiar feature of *C. Melolonthae* is the development of the head from two or more separate areas near the apex of the clava. The perithecia are not embedded in the original clava, but in a superposed thickness of tissue which resembles a parasitic *Hypocrea*. These areas extend laterally until they ultimately coalesce. The appearance of the head will therefore depend upon the stage of development reached when the specimen is collected. But when the perithecial tissue has become continuous, there is usually a groove, with a V-shaped opening at the base, down one or both sides of the head.

It is to be noted that this development of the head from two or more lateral areas is similar to that of *Cordyceps Volkiana* Möller, found on lamellicorn larvae in Brazil. The clavae of Möller's specimens are more irregular, and in the most regular one the stalk bifurcates above and each branch bears a separate head, but it must be remembered that these clavae were developed under damp conditions, "in eine feucht gehaltene Schale," in the laboratory, and are therefore most probably abnormal. It would only require a bifurcation of the stalk of *C. Melolonthae* to produce *C. Volkiana*.

C. Cusu was described by Patouillard from specimens collected by Lagerheim in Ecuador. The specimens were immature. In Herb. Berlin, there are similar specimens from Peru, to which is attached the native name "El Cusso ó Cuzo." The latter were assigned by Hennings to *C. Melolonthae*, and it is probable that they are that species.

As already noted, Lloyd referred *C. insignis* Cke. & Rav. to *C. Melolonthae*. Ravenel sent *C. insignis* to Cooke at Kew, with a note stating that he had found only one specimen, which he had divided into two, forwarding half the fungus and the whole of the large larva. In accordance with that, the other half of that specimen is now in Herb. British Museum, *ex* Herb. Ravenel. But the sheet in Herb. Kew. bears two specimens, and it is evident from Cooke's description that he had two specimens when he described it.

Ravenel's note accompanying the specimens in Herb. Kew. is, "I found but a single example of this and have divided the stipe and capitulum, retaining half. I send the whole of the large larva. The colour is pretty well preserved. I have seen several insect *Cordyceps* here, but this differs from anything I have seen. On dead larva buried in the ground. Seabord of S.C., April 1881. H. W. Ravenel." It is to be regretted that Ravenel did not record what the colour was when fresh, as Cooke did not publish a description until two years later.

On the half specimen in Herb. British Museum, Ravenel's label, as far as it is decipherable, is "*Cordyceps insignis* Cke. and Rav., on dead larva buried in the ground, near. . .ditch, St John's, . . ., S.C., H. W. R."

The sheet in Herb. Kew. contains the half specimen of the fungus, an unattached larva, straight and cylindric, about 2 cm. long and 8 mm. diameter, which is probably the one referred to by Ravenel, and another specimen of the *Cordyceps* with the larva attached. Cooke drew his figure of the fungus from the half specimen, which may be taken as the type. His description covers both specimens.

The half specimen has an obovate or pyriform head, broadest above, about 11 mm. high and 8 mm. broad at its widest part, slightly rough with the apices of the perithecia. The head descends farther down the stalk on one side than on the other and has a median V-shaped groove in its lower edge, so that it may be described as bilobed at its junction with the stalk. The half in Herb. British Museum, which of course shows the other face of the head, is similar. The stalk is about 2 cm. long and 3 mm. broad, and divides at its base into cords of mycelium which become narrow flattened ribbons.

The unattached larva is completely covered by a yellowish film of mycelium, and no segments are evident, nor is the head visible.

The second specimen is remarkably different in appearance. Its

head is irregularly subglobose, or laterally oval, about 1 cm. high and 2 cm. diameter. The stalk is almost wanting, the head being attached to the larva by a twisted stalk-like mass of flattened strands of mycelium. The larva is about 3 cm. long and 1 cm. diameter, but part of the hinder end has been cut off; it is slightly curved, and, like the other specimen, it is covered with a uniform sheet of mycelium which hides the dividing lines of the segments and the head. The strands of mycelium which unite the *Cordyceps* to the larva arise round the head from the sheet of mycelium. On laying bare the anterior end of the larva, it was determined that it was probably a beetle larva, but not a cockchafer.

The two specimens are the same species. The perithecia are wholly immersed, and in section the ostiola barely project. The perithecia are narrow flask-shaped, 0·7 mm. high, 0·25 mm. diameter, now reddish brown above and brownish yellow below. The neck of the perithecium is about 0·1 mm. diameter, but it expands above and becomes about 0·25 mm. diameter with a convex apex. The *Cordyceps* head has a continuous cortex which is composed of the fused apices of the perithecia with very little intermediate tissue. Consequently, it appears in section as composed of agglutinated parallel hyphae, more or less perpendicular to the surface. The part-spores are cylindric with rounded ends, 4–9 × 1–1·5 μ. The context of the head is white or yellowish white.

Cooke's figure in *Vegetable Wasps*, etc., Pl. I, fig. 3, shows the lower edge of the head straight, and in that respect is incorrect. It also shows a solid stalk arising from the larva, which is not in agreement with the specimens. Cooke evidently drew his figure of the fungus from the half specimen, and the larva from the other specimen. His figure shows the segments of the larva, which on the specimen are hidden by mycelium, and a series of triangular processes on the under side. The only justification for these apparent legs is a number of scale-like fragments of wood, about 2 mm. long and 0·5 mm. broad, which are loosely attached to the larva and to one another by fine yellow hyphae. As these fragments occur on both specimens, it may be suggested that the larva is one which surrounds itself with fragments of wood before pupation. When these fragments are cleared away, the larva appears as a uniform mummy without any indication of legs, segments, etc.

Cordyceps insignis is not *C. Melolonthae*. In cockchafer larvae attacked by a *Cordyceps*, the fungus arises in one or more stout clavae, generally, though not always, just behind the head. The larva is usually not covered, or only slightly covered by mycelium, and the segments, legs, and horny parts of the head are plainly visible. In *C. insignis*, the larva is completely hidden by mycelium and is attached to the *Cordyceps* by strands of mycelium.

Lloyd was probably influenced in his decision by the presence of a V-shaped groove at the base of the head on either side. But that is the only resemblance. *C. insignis* has a continuous cortex and its perithecia are embedded in the tissue of the head. *C. Melolonthae* is of the intermediate type (*Trans. Brit. Mycol. Soc.* XVIII (1933), 51); there is no definite cortex, the tissue between the perithecia is loose and friable, and in section the perithecia appear to be seated on a central core.

Further, the perithecia in a South American specimen of *C. Melolonthae* were ovoid or conoid, 0·25–0·3 mm. high, 0·12–0·16 mm. diameter, less than half the size of the perithecia of *C. insignis*.

Cooke described *C. insignis* as purple. If that is correct, the two species differ in colour.

C. Melolonthae appears to be confined to the Western Hemisphere. There is a record of it for Australia by Oliff, which, as Lloyd remarked, appears to be based on "very uncertain material." In the Eastern Hemisphere, the corresponding *Cordyceps* on cockchafer larvae is *C. Barnesii* Thw., which has a much longer, narrower stalk and a smaller head, with a conical sterile apex which may bear the conidial stage, a *Stilbella*. *Cordyceps Barnesii* shows affinity with *C. Melolonthae* in that the fertile portion of the head looks like an extra layer wrapped round the stalk, and it is sometimes grooved, or has a V-shaped opening, on one side. *C. atrobrunnea* Penz. & Sacc., said to be on a Lepidopterous larva, and *C. Fleischeri* Penz. & Sacc., both from Java, appear from the figures and descriptions to be *C. Barnesii*, while *C. obtusa* Penz. & Sacc., also from Java, on beetle larvae, differs from *C. Barnesii* in having the head continuous over the apex.

I am indebted to Mr E. H. Ellis for the photograph of *C. Melolonthae*.

80. *Cordyceps Humberti* Robin

In *Monographie des Guêpes Sociales* (1853–8), H. Saussure figured (Pl. V, fig. 9) a *Cordyceps* on *Vespa cincta* from Senegal, which had been named *Cordyceps Humberti* by Robin. He gave a brief account of the fungus, but apparently Robin did not publish a formal description.

Tulasne (*Sel. Fung. Carp.* III (1865), 18) referred the fungus to *Torrubia*, as *T. Humberti*, and gave a description, probably drawn up from Saussure's figure, which is that entered in Saccardo, *Sylloge Fungorum*, II (1883), 576.

The insect bore two perithecial clavae, one from the base of the wings on each side, and several longer and more slender clavae from the sutures of the abdomen. The heads of the perithecial clavae were oval, and rough with projecting ostiola. Cooke's reproduction of the figure omits the details over the surface of the head, merely giving the outline. Two of the slender clavae from the abdomen had minute oval

tips, which were regarded as immature perithecial heads. Gray, as cited by Cooke, stated that these tips were "apparently trifoliated," but in the figure they are simply oval or lanceolate. This suggests that Gray may have seen another figure.

Saussure also gave an illustration (Pl. XI, fig. 5) of another fungus on *Polistes americana*. This fungus had been previously recorded by Felton in 1765 on the same insect (*Phil. Trans.* LIV, 54, Pl. 6). But Felton regarded the fungus as part of the wasp, which he named *Vespa crinita*. Cooke (*Vegetable Wasps*, etc.) named the fungus *Isaria Saussurei*, and it now stands as *Hirsutella Saussurei* (Cke.) Speare.

Tulasne noted that *H. Saussurei* appeared to be related to *Cordyceps Humberti* rather than to *C. sphecocephala*.

I have examined a specimen of *C. Humberti*, on a Hymenopteron, from Sarawak, collected by the Oxford University Expedition to that country in 1932. There were two clavae from the thorax, one from the points of insertion of the wings on each side. One of these was broken. The other was dark brown, 7 mm. long, with an oval swelling, 1 mm. long, 0·4 mm. diameter, about half way up. The stem was slightly thicker below the swelling than above, the upper part being hair-like, tapering to the apex. The swelling is the head, which is not terminal, but not lateral as in *Ophiocordyceps unilateralis*. The head is rough with strongly projecting ostiola, the latter being dark amber, subtranslucent, flask-shaped with a truncate apex, projecting up to 275 μ, and up to 120 μ diameter at the base. The head contained only a few scattered perithecia, and one isolated perithecium occurred on the stalk below the head. The asci are 130 μ long, 10 μ diameter, capitate, fusoid or narrow-clavate, the spores in a parallel bundle, but the individual spores not reaching both ends of the ascus. The spores are 75 μ long, 2·5 μ diameter, narrow-fusoid, septate at intervals of 6 to 16 μ, not dividing into part-spores. The fungus is an *Ophiocordyceps*.

Three dark brown, hair-like clavae arose from the last suture but one of the abdomen. These were *Hirsutella Saussurei*.

One frequently finds a minute apical swelling on the clava of *H. Saussurei*. These may be oval or conical, and are sometimes constricted about half-way up, so that in longitudinal section they would appear trifoliate. But they are not represented so in Saussure's figure of *Cordyceps Humberti*. I have always found these tips sterile. It is doubtful whether they are immature perithecial heads as the latter would appear to be always formed in the middle of the clava. In Saussure's specimen the apices of the perithecial clavae had probably been broken off.

Cordyceps myosuroides P. Henn., described from a specimen said to be on an Ichneumon from Brazil, is the same species. The host insect is a wasp. Of the two perithecial clavae on the type specimen, one is

immature, and the head is developing some distance below the tip, leaving a long thin apex, while the other is mature, with apparently a terminal head, but examination shows that the upper part of the clava has been broken off. The head is rough with strongly projecting perithecia, which Hennings described as two-thirds free.

Cordyceps goniophora Speg. appears, from the description, to be another synonym of *C. Humberti*. It was described by Spegazzini from a specimen on *Mutilla* sp. The clavae were said to be brownish black, setiform, rigid, subhorny, 5–20 mm. long, 0·1–0·3 mm. diameter, generally with an obovate or obclavate swelling, 1 mm. long, 0·5 mm. diameter, about the middle. The clavae were immature and did not bear perithecia. Spegazzini regarded the swellings on the clavae as old heads which had emitted new growths from their apices. The position of the swellings, however, is normal for the heads of *Cordyceps Humberti*, and there does not appear to be any doubt that he had an immature example of that species.

C. sphecocephala (Kl.) Cooke, the best known *Cordyceps* on Hymenoptera, has immersed perithecia, ascospores which divide into part-spores, and a *Hymenostilbe* conidial stage. *Cordyceps Humberti* has strongly projecting perithecia, ascospores which do not divide into part-spores, and a *Hirsutella* conidial stage. The synonymy of the latter, as far as has been ascertained at present, is as follows:

Ophiocordyceps Humberti (Robin) Petch, comb.nov.; *Cordyceps Humberti* Robin in Saussure, *Mon. Guêpes Soc.* (1853–8), pp. clxiv and 39, Pl. V, fig. 9; *Torrubia Humberti* (Robin) Tul., *Sel. Fung. Carp.* III (1865), 18; *Cordyceps goniophora* Speg. in *Bol. Acad. Nac. Cienc. en Cordoba* (*Republica Argentina*), XI (1889), 537; *Cordyceps myosuroides* P. Henn. in *Hedwigia*, XLI (1902), 169. Conidial stage, *Hirsutella Saussurei* (Cke.) Speare in *Mycologia*, XII (1920), 69; *Isaria Saussurei* Cooke, *Vegetable Wasps*, etc. (1892), p. 53; *Isaria crinita*, Lloyd, *Mycol. Notes*, No. 54 (June, 1918), p. 778, fig. 1173.

81. *Cordyceps Puiggarii* Speg.

In *Fungi Argentini*, Pugillus IV, No. 207, Spegazzini recorded *Cordyceps sphecocephala* on *Polybia fasciata*, and gave a re-description which was included in Saccardo, *Sylloge Fungorum*, II (1883), 568. Subsequently Spegazzini decided that his fungus was not *Cordyceps sphecocephala*, and in *Fungi Puiggariani*, Pugillus I (1889), 157, he described it as *C. Puiggarii* n.sp. He stated that the head was attenuated into a rather long, acute, conical, sterile apex. That, however, is not uncommon in *C. sphecocephala*, and the other details given by Spegazzini do not show any differences from that species. Its perithecia were said to be "immersa, vix prominula"; hence it cannot be referred to *C. Humberti*. The name *Cordyceps Puiggarii* Speg. was duly entered in Saccardo, *Sylloge Fungorum*, IX (1891), 1000.

In 1919 (*Bol. Acad. Nac. Cienc. Cordoba*, XXIII, extr. p. 116), Spegazzini described another species as *Cordyceps Puiggari*. This was on a beetle, *Lystronychus* sp., and from the description it would appear to be *Cordyceps curculionum* (Tul.) Sacc.

82. *Cordyceps* on Orthoptera

The species of *Cordyceps* recorded on Orthoptera include *C. locustiphila* P. Henn., from Brazil, *C. amazonica* P. Henn., from Brazil, and *C. Uleana* P. Henn., from Peru, all described in 1904, and *C. stiphrodes* Syd. and *C. ctenocephala* Syd., from New Guinea, described in 1922. The last-named was said to be on a Cicada, but it would appear to be on a Locustid.

C. locustiphila differs from *C. amazonica* in the shape of the head. In the type of the former, the clavae are curved from beneath the insect and terminate in laterally compressed, clavate heads, about 8 mm. long, 2 mm. diameter. In *C. amazonica*, the clavae are short and erect, and the heads are ovoid, about 4 mm. high, 3·5 mm. diameter, contracted abruptly into the stalk. In both, the heads are minutely rough with red-brown, slightly projecting ostiola, with a pale brown, detersive pruina between the ostiola, and the perithecia are immersed. The two agree in all essential details, and *C. amazonica* must be regarded as a synonym of *C. locustiphila*. I have a specimen from Trinidad, on a mole cricket, in which the head is globose.

Judging from the figure and description, *C. stiphrodes* Syd. is *C. locustiphila*.

The type of *C. Uleana* has clavae with a short stout stalk and a globose head with strongly projecting perithecia. I have been able to examine two other specimens of this species from Madagascar, one mature and the other immature, on Phasmids. According to the collector's note, the colour when fresh was pale yellow. The immature clavae are now white, up to 4 mm. high, usually simple, sometimes concrescent at the base, with a stalk up to 0·5 mm. diameter below, usually expanding upwards and terminating in a globose or ovoid head, up to 1 mm. diameter, dotted with minute yellow-brown points which are the initials of the perithecia. In the mature specimen, the heads are up to 1·5 mm. diameter, strongly echinulate, and consist of a group of strongly projecting perithecia, with mycelial tissue between their bases. The perithecia are now dark amber, tomentose below, conoid with an acute apex, up to 0·4 mm. high, 0·25 mm. diameter, projecting three-quarters of their height. The asci are cylindric, capitate, 4μ diameter, and the ascospores linear, 1μ diameter, in a parallel bundle. The part-spores are cylindric, 3–9 × 1μ. *C. Uleana* resembles the form of *C. tuberculata* (*C. Sphingum*) which Curtis named *C. isarioides*.

In Herb. Kew., there is a specimen of *C. Uleana*, collected by Traill in Brazil in 1874. It is included in the cover of *C. Sphingum*, and is the basis of Cooke's statement that *C. Sphingum* "is said to occur also on *Orthoptera*," and Massee's later record of that species on a small Orthopterous insect. The insect bears slender clavae, each bearing superficial, scattered or clustered perithecia. A similar specimen, now in Herb. Berlin, was collected by Tessmann in Peru, and I have examined another, with immature clavae only, from British Guiana (coll. E. B. Martyn).

C. ctenocephala Syd., judging from the figure and description, is *C. Uleana*.

Lloyd (*Mycol. Notes*, No. 62 (1920), p. 913, fig. 1622) suggested that *C. Joaquiensis* P. Henn., recorded by Hennings with a query as on a Coleopterous larva, was on a mole cricket, as he had received specimens on mole crickets which resembled Hennings's figure, and the host in the figure appeared to him to be a mole cricket. But the figure shows the host completely enveloped by mycelium, and its nature must remain uncertain, until the type specimen is re-examined.

83. *Cordyceps sobolifera* (Hill) Sacc.

The fungus which grows on Cicada nymphs, and is now known as *Cordyceps sobolifera*, has been the subject of numerous articles, the relevant data from which were republished by Cooke in his *Vegetable Wasps and Plant Worms*. It was named *Clavaria sobolifera* by Hill, in *An account of the insect called the Vegetable Fly* by W. Watson in *Phil. Trans. Roy. Soc.* LIII (1763), 271–4, with a figure. Hill had specimens from Martinique, and Watson from Dominica. A few years later, Fougeroux de Bondaroy contributed a paper, *Sur les insectes sur lesquels on trouve des plantes*, to *Hist. l'Acad. Roy. des Sciences, Paris*, année 1769 (1772), pp. 467–76, Pls. 4, 5, in which he included the Cicada fungus without stating the origin of his specimens, though they were no doubt from the West Indies. Cooke (*op. cit.*) reproduced three figures from Fougeroux de Bondaroy, but he turned one of them (Cooke, fig. 45) through 90°.

Cordyceps sobolifera owes its name to the fact that Hill's specimen bore lateral, more or less oval outgrowths (soboles) about the middle of the stem. These outgrowths are conidial, and are apparently easily detached, so that the mature *Cordyceps* may not be soboliferous. The early figures show usually a lobed head, or more rarely a simple cylindric head, with or without soboles on the stem.

The conidial stage of *Cordyceps sobolifera*, *Isaria Cicadae* Miq., usually has a much branched and lobed head, the lobes being powdery with conidia, but it may have a head of few lobes, in which the lobes are even and compact, appearing at first glance to be perithecial clavae.

Judging from the figures, Watson's specimen, and those figured by Fougeroux de Bondaroy, were conidial clavae. Fig. 7, Plate 4, of the latter writer, which shows a long cylindrical head, may represent a perithecial clava, but he did not state that it differed in texture from the others.

The first formal description of *Cordyceps sobolifera* was published by Berkeley in 1843 in *Hooker's London Journal of Botany*, II, 207, under the name *Sphaeria sobolifera*. He described it as "carnosa, pallide fusca; capitulo subgloboso, stipite aequali tereti prolifero." Berkeley did not cite any type specimen, but stated that there were several specimens in the herbarium of the British Museum. There are two old specimens now in Herb. British Museum, marked "Vegitans, W. I.," without date. In both, the stalk is simple, without processes. In one, the head is elongated oval, 7 mm. long and 4 mm. diameter, and the stalk about 2 cm. long, 2 mm. diameter in the middle, attenuated above and below. The other is apparently immature, and has a cylindrical head, 6 mm. long, 2 mm. diameter, and a stalk about 3 cm. long, 2 mm. diameter in the middle, not noticeably attenuated above and below. The head is sharply defined from the stalk, and terminates in an even line round the latter.

Fig. 2. *Cordyceps sobolifera*, from Vegitans, W. I. × 1½.

In all the undamaged specimens of *Cordyceps sobolifera* which I have seen, the head is oval, or cylindric, or subglobose, and terminates evenly below, while the stalk is cylindric and does not bear soboles. A specimen from Madagascar, in Herb. Berlin (*C. Voeltzkowii* P. Henn.), is accompanied by a drawing which shows soboles on the stem, but there are none on the specimen now. On the other hand, a Ceylon specimen did not bear any soboles when collected. The name, *C. sobolifera*, is unfortunate, as it would appear that the perithecial clava either does not bear soboles, or if it does they are not persistent.

The Tulasnes included *C. sobolifera* (as *Torrubia*) in *Selecta Fungorum Carpologia*, III (1865). They stated that the head was ovato-oblong or linguaeform, with a thick, terete, rigid, erect, glabrous and naked stalk, but sometimes the stalk bore a few short branches, either crowded in the middle, or remote and alternate. It would appear that they deduced the last statement from the literature, as they stated that in the specimens they had from Martinique, the stalks were simple and naked. The soboles are scarcely branches of the stem, but oval bodies loosely attached at their bases.

The Tulasnes gave a figure showing the whole fungus attached to the insect, and, as that specimen was immature, they supplied a drawing of part of an ascus with part-spores from another specimen. Their figure created some difficulty, as it differs in an important feature from the available specimens. It shows, down the head, a longitudinal groove which opens out into a V-shaped gap at the base, whereas on recent specimens the head is uniformly terete and the lower edge is even. The figure, indeed, more closely resembles some specimens of *C. Melolonthae*, in which species the longitudinal groove and V-shaped opening are a consequence of the peculiar mode of development of the head, and it is interesting to note that Cooke (*Vegetable Wasps*, etc., p. 96, footnote) cited the Tulasnes' figure as an illustration of *C. Melolonthae*.

Fig. 3. *Cordyceps sobolifera*, from Madagascar. ×⅔.

Lloyd pointed out that the specimen from which the Tulasnes' first figure was drawn is still in the Paris herbarium, and, through the courtesy of the officers in charge, I have been permitted to examine it. The specimen agrees with the figure, but it is evident that the groove is accidental and due to pressure or shrinkage in drying. The head is flattened, and one side is grooved as shown in the figure, while on the other side the groove begins at the apex, but does not extend to the base.

The specimen recorded from Ceylon as *C. gracilis*, and figured in *Trans. Brit. Mycol. Soc.* x (1924), Pl. I, fig. 2, is *C. sobolifera*. The record of *C. sobolifera* on cockchafer larvae, in *Fungi of Ceylon*, No. 978, is, as previously noted by Massee, an error, the fungus being *C. Barnesii*. The latter record is the basis of the statement in Saccardo, *Syll. Fung.* II (1883), 569, that *C. sobolifera* occurs on Lamellicorn larvae.

Massee (*Ann. Bot.* IX (1895), 13) recorded *C. sobolifera* on the larva of a beetle, probably one of the Melolonthidae, South America, the specimen having a note on the label, "on a larva which destroys the cotton crop in S. America." This specimen is still in Herb. Kew., but it is not *C. sobolifera*.

As already indicated, *C. Voeltzkowii* P. Henn., from Madagascar, is *C. sobolifera*.

C. sobolifera is known, in its perfect stage, from the West Indies,

Japan, Ceylon, and Madagascar. The conidial stage is known also from Mexico, China, and New Zealand.

In colour, *C. sobolifera* is at first white with a pinkish tinge, becoming brown. In dried specimens, the stalk is pale brown to rufous brown, and the head red-brown with darker ostiola. In a collector's note on a Madagascar specimen, the fungus is said to be red-brown. The total height in the available specimens varies from 2·5 cm. to 11 cm. The stalk is from 2 cm. to 9·5 cm. long, terete, sometimes equal, sometimes inflated in the middle. A large specimen from Madagascar has a stalk 2·5 mm. diameter below, expanding to 4 mm. in the middle and diminishing to 3 mm. at the apex. The stalk is glabrous and even when fresh, but may become longitudinally furrowed in drying. The head is usually sharply defined from the stalk when fresh, but it may not be so distinct on dried specimens; it may be cylindric, oval, or subglobose, up to 1·8 cm. high and 9 mm. diameter. On large specimens the head is small in comparison with the stalk. The perithecia are immersed, and on a fresh specimen the ostiola were not evident, but on herbarium specimens the head is rough with slightly projecting ostiola, darker than the head. The perithecia are flask-shaped with a rather long neck, 0·4–0·6 mm. high, 0·3 mm. diameter.

In a Ceylon specimen, the asci were 8μ diameter and the part-spores cylindric, 4–8 × 2μ. Patouillard has noted the breadth of the ascus as 8μ, and the length of the part-spores as 7–8μ, on a specimen from Madagascar in Herb. Paris. Hennings stated that the ascospores of *C. Voeltzkowii* were 1·5–2μ diameter, and Lloyd (*Mycol. Notes*, No. 42 (1916), p. 584) gave the part-spores as 8–12 × 1μ in a specimen from the Bahamas.

I am indebted to Mr E. H. Ellis for the photograph of *C. sobolifera* from the West Indies.

84. *Cordyceps Stenocori* Quélet

Cordyceps Stenocori was described and figured by Quélet in *Assoc. Franç. pour l'Avanc. d. Sci.* XXIV (1895), 622, Pl. 6, fig. 18. It occurred on a beetle, *Stenocorus mordax* Fab. From the figure and description, it was evidently a conidial form only.

From Quélet's remark, that it appeared to be very near *C. militaris*, one might deduce that it was the form of *Spicaria* (*Isaria*) *farinosa* which occurs on beetles, but the description of the conidia as ellipsoid, 5μ, bi- or tri-guttulate, is against that supposition. The figure resembles some forms of *Hirsutella Eleutheratorum*, but does not show the seta-like phialides. The conidia are figured as oval, with a well-marked cylindric apiculus. Until the type is re-examined and the conidiophore determined, this species cannot be classified, except indefinitely as *Isaria*.

85. *Stereocrea coccophila* Petch, n.sp.

This species occurred on a scale insect on *Eugenia*, at Nuwara Eliya, Ceylon, January 16th, 1927.

The stromata are at first flattened pulvinate, up to 4 mm. diameter, 1·25 mm. high, completely covering the scale. Ultimately they become irregularly tuberculate, up to 7 mm. diameter, with flattened turbinate tubercles, the convex upper surface of which is oval in plan and up to 2·5 × 1·5 mm., or larger by confluence. At first they are red-brown, but become black with a greyish bloom. Internally they are orange-yellow. Alcohol gives a yellow extract, which turns yellow-brown with caustic potash. The context is rather soft and crumbly, and consists of stout, thick-walled hyphae, with deposits between them as in *Myriangium*. In the young pulvinate stroma, there is a well-marked cortical layer of radial parallel cells, and in the centre of the stroma, chains of cells not united laterally. In the older stromata, the cortex is stout and black, but the cells are not radial. The blackening is due to a blackish green deposit between the cells. The cortex breaks up when old, but it is not carbonaceous.

The perithecia are situated in the tubercles, none having been observed in the merely pulvinate stromata. They are irregularly placed, sometimes distant, sometimes crowded, peripheral, usually in a single layer, 0·4 mm. high, 0·25–0·3 mm. diameter, subglobose or ovoid, with a short conical neck. The perithecial wall is yellow, about 30–40μ thick, composed of parallel slender hyphae. The ostiola are not evident externally. The perithecia are polyascigerous, the asci being clavate, 140μ long (including a pedicel about 40μ long), 24μ diameter, soon diffluent, eight-spored, the spores being obliquely uniseriate below and biseriate above. The apex of the ascus is not thickened and does not turn blue with iodine. There are no paraphyses. The ascospores are cylindric with rounded ends, hyaline, 3 to 5 septate, not or slightly constricted, minutely warted, with a hyaline mucous coat, 32–50 × 10–12μ.

The general appearance and structure of the stroma are those of *Myriangium*. The loculi, however, are ostiolate and polyascigerous.

Stereocrea coccophila Petch, n.sp.—Stromatibus immaturis depresso-pulvinatis, usque 4 mm. diam., 1·25 mm. alt., dein irregulariter tuberculatis, tuberculis confertis, depresso-turbinatis, supra convexis; extus primo rufo-brunneis, dein nigris, intus aurantiacis; peritheciis in tuberculis sitis, peripheric is, saepius monostichis, confertis vel sparsis, subglobosis vel ovoideis, 0·4 mm. alt., 0·25–0·3 mm. diam., collo conico brevi, omnino immersis, ostiolis non emergentibus, pariete flavo, 30–40μ crasso, ex hyphis tenuissimis dense parallele dispositis; ascis clavatis, 140 × 24μ, mox diffluentibus, octosporis;

sporis cylindraceis, utrinque rotundatis, hyalinis, 3–5 septatis, non vel leniter constrictis, minute granulatis, muco hyalino indutis, 32–50 × 10–12 μ.

On a scale insect on *Eugenia*, Nuwara Eliya, Ceylon.

86. *Entomophthora Aphrophorae* Rostrup

Entomophthora Aphrophorae was briefly described by Rostrup in 1896 (*Botan. Tidskr.* xx, 128, with figure). It occurred on *Aphrophora spumaria* (= *Philaenus spumarius*), a froghopper, in Denmark. He stated that, when fully developed, it was of a bright orange colour.

Specimens were collected on *Philaenus spumarius* on bramble at Arncliffe Woods, near Whitby, in August, 1930 (F. A. Mason), and at Grassington in September, 1931, but owing to their condition when received or collected they were passed over as *Entomophthora sphaerosperma*. In September, 1933, a specimen on *Philaenus* sp. was received from Fritton Bog, Suffolk, while it was collected in quantity at Aldbrough and Hedon, East Yorks., and in smaller numbers at Lartington and Deepdale, North-West Yorks., and at Dipton Wood near Corbridge. The majority of the specimens occurred on the under side of leaves of *Ranunculus repens*, but it was found also on *Plantago lanceolata*, *Carduus arvensis*, *Juncus*, etc.

The insect is attached to the leaf by fascicles of coarse brownish rhizoids, which are not expanded or lobed at the extremity, from the under surface of the body. The conidiophores form a stroma which appears round the insect and along the back between the wings, and ultimately extends so as to cover the whole insect, except for two parallel, longitudinal, oval areas, one over the middle of each wing. The fully developed stroma is remarkably thick, compact, waxy-looking, viscid, at first yellow or greenish yellow, becoming orange or reddish. Cystidia were not observed. Although the stroma is exceptionally thick, it rapidly shrinks, after maturity, to a very thin translucent film, and specimens in the latter condition could not be identified correctly without a previous knowledge of the fungus.

The conidia are narrow oval, with a convex papilla occupying the whole base and defined by a collar. They are sometimes surrounded by a hyaline mucous coat, thickest at the apex (3 μ) and continuous or discontinuous along the sides, but not extending over the base. Their contents are granular, sometimes with a central nuclear body. On dried specimens from Arncliffe Woods (1930), the conidia found measured 20–24 × 6 μ, and on similar specimens from Grassington (1931) they were 21–30 × 7–9 μ, while in a spore print obtained from fresh specimens at Hedon in 1933, they were 20–30 × 7–10 μ, usually about 27 × 8 μ. Rostrup gave the dimensions as 16–18 × 7–8 μ. Compared with *Entomophthora sphaerosperma*, the conidia are longer and more regularly oval.

Germination occurs from the middle of one side of the conidium. The secondary conidia are either obpyriform, curved at the apex, 12–18 × 7–9μ, on a slender conidiophore, up to 60μ long, 2μ diameter at the base, tapering to 1μ above, or broadly oval, 12–20 × 8–9μ, apex usually acuminate, on a stout conidiophore, 9μ long, 6μ diameter.

The resting conidia (azygospores) are spherical, hyaline, thick-walled (3μ), 25–33μ diameter.

E. Aphrophorae differs from *E. sphaerosperma* in the consistency of its stroma, in its longer and more oval conidia, and in the shape of the secondary conidia.

87. *Empusa Muscae* Cohn

This species is usually recorded as occurring on house flies attached to window panes in the autumn. In my experience, it is much more common out-of-doors during the summer months, when it may be found on flies of several kinds, but especially on "dung flies" (*Scatophaga* spp.) on the flower heads of grasses, *Juncus*, *Rumex*, *Senecio*, and Umbelliferae. In 1933, I received specimens on dung flies collected in June in Oxfordshire, Staffordshire, and Surrey, while in July, 1931, I saw hundreds of examples, chiefly on dung flies, in East Yorkshire. It is most abundant on marshy ground, especially in wet seasons, but examples may be found at random over the country every year.

The dung flies are not attached to the plant by their probosces, as are the flies on a window pane, but merely clasp the inflorescence with their legs.

One point in which the *Empusa* on the dung flies appeared to differ from *Empusa Muscae* was the presence of a rather broad longitudinal band of conidiophores forming a stroma along each side of the abdomen on the lower surface. This raised doubts about the identity of the species, as, according to all accounts, *E. Muscae* does not form a continuous stroma over the body of the fly. In most species of Entomophthoraceae on flies, the conidiophores first appear along the sutures, and subsequently spread and coalesce, covering the whole of the body, except sometimes the centre of the thorax. In *E. Muscae*, however, the conidiophores remain restricted to the sutures, so that the abdomen is encircled by white rings. But on examining a specimen of *E. Muscae* on a house fly, which had died in the classic manner on a window pane, it was found that it had two narrow longitudinal continuous bands of conidiophores along the under surface of the abdomen. There was therefore no difference between the distribution of the fungus on *Scatophaga* and that on *Musca*, the greater development of the longitudinal bands on the former being probably due to a more humid environment in the open.

While it is correct that the conidiophores of *Empusa Muscae* do not

coalesce *over* the body of the insect, they do coalesce along its sides or under it.

It is probable that *Empusa Muscae* on *Scatophaga* is what was named by Giard, *Entomophthora Scatophagae*, without description. Giard stated that it resembled *Empusa Muscae*, but differed from that species in its larger yellow conidia. The conidia on *Scatophaga* do appear yellowish in mass on dried specimens, though not when fresh, but they are within the range of dimensions of *Empusa Muscae*. Giard also stated that the dead insect was fixed by its feet and abdomen, from which Guéguen deduced that *Entomophthora Scatophagae* possessed rhizoids, but the deduction does not appear to be warranted. The feet of the insect are entangled in the inflorescence of the plant.

Entomophthora Syrphi Giard (*nomen nudum*) would appear to be also *Empusa Muscae* out-of-doors.

I have not seen any record of *Empusa Muscae* except on flies. In August, 1933, Mr F. T. Brooks sent me from Cambridge a capsid bug (*Lygus pabulinus*) which had attracted attention by its enormously distended abdomen. Sent in a tin box, it died in transit and developed typical *E. Muscae*. The primary conidia were the normal shape, and the secondary conidia were the same shape or oval. The specimen, therefore, could not be assigned to *Empusa erupta* Dustan, which was described from *Lygus communis* var. *novascotiensis* in North America.

88. *Aschersonia caespiticia* Syd.

Aschersonia caespiticia was described by Sydow in *Engler's Bot. Jahrb.* LIV (1916), 260, from specimens from New Guinea. Through the kindness of Dr Sydow, I have recently received part of the type specimen.

The stromata are up to 3 mm. diameter, pulvinate, covered with ovate or subcylindric processes. The *Aschersonia* pycnidia are situated near the base of the stroma, and are completely immersed, oval in plan, but with the sides convoluted, so that the pycnidia appear labyrinthoid in section. The ostiolum is small, and does not project, and the pycnidium communicates with the exterior through a tube of varying length. The pycnospores are narrow fusoid, $9–13 \times 1–1{\cdot}5 \mu$, rarely 2μ broad, and there are no paraphyses. The fungus is on a *Lecanium*.

This is the *Aschersonia* stage of *Hypocrella Amomi* Rac. The projections are the perithecial processes, but in the specimen examined they were solid and immature. The entire fungus agrees with the figure of *H. Amomi*, *Ann. Perad.* VII (1921), Pl. II, fig. 5. In the type specimen of *H. Amomi*, the perithecia and the pycnidia occurred in separate stromata. In the present collection, they are combined in the same stroma, but the perithecial processes are immature.

89. *Patellina epimyces* Petch, n.sp.

This species was collected at Peradeniya, Ceylon, in May, 1921, growing on a brown or greyish brown stroma on a froghopper on mango leaves. This stroma, which covers the insect almost or completely, is frequent at Peradeniya, but is generally sterile. It has recently been determined as that of *Hirsutella versicolor*, a species which is usually fertile when found at higher elevations.

The same *Patellina* was collected by Miss E. M. Wakefield in Trinidad in 1921, parasitic on *Hirsutella entomophila* Pat. In the Ceylon specimens, which occur on the even continuous stroma covering the insect, the sporodochia are flat and saucer-shaped, but in the Trinidad specimens, in which they are seated on the very slender clavae of *H. entomophila*, they are obconic.

Patellina epimyces Petch, n.sp.—Sporodochiis discoideis, rotundatis vel lobatis, usque 0·5 mm. diam., 0·1 mm. crassis, vel obconicis, usque 0·15 mm. diam., 0·1 mm. alt.; disco aurantiaco-rubro, ceraceo, margine albo pruinoso; extus albis, tomentosis, ex hyphis parallelis supra clavatis, 2·5–4 μ diam., minute verrucosis, compositis; conidiophoris confertis, ampullaceis, 9 μ alt. infra 2 μ diam., ad 0·5 μ diam. supra attenuatis; conidiis angusto-ovalibus, utrinque acutis, hyalinis, 2–4 × 1–1·5 μ.

Parasitic on *Hirsutella versicolor* Petch, Peradeniya, Ceylon, and on *H. entomophila* Pat., Trinidad.

90. *Hirsutella entomophila* Pat.

This species which occurs on beetles was described by Patouillard in *Rev. Myc.* 1892, p. 69, as having rigid simple clavae. A number of specimens were collected by Dr C. B. Williams at Mareval, Trinidad, in 1916, and others by Miss E. M. Wakefield at Moruga, River Estate, and elsewhere in the same country in 1921. Some of these are more developed than the specimens described by Patouillard, and are extensively branched.

The clava is up to 1 cm. high. It consists of an erect, rigid, hair-like main stem, about 0·1 mm. diameter at the base, which divides only near the apex, if at all. From this stem, primary lateral branches, about 0·04 mm. diameter, up to a dozen or more, somewhat distant from one another, spread horizontally, and these bear secondary lateral branches, about 0·02 mm. diameter, which extend horizontally and vertically. Branching is always practically at right angles, and the branches are usually straight and rigid, but may be curved at the tips. The fungus thus forms a tree-like structure. Several of these clavae may arise from the body of the beetle, and others from brown strands of mycelium which run from the insect over the surface of the leaf to which it is attached, as far as 2 cm. from the insect.

These dendroid clavae suggested comparison with *Isaria ramosissima* Zoll. & Mor., found on the elytra of a beetle in Java, and described in *Natuur- en Geneeskundig Archief voor Neërland's-Indie*, 1 (1844), 376. The original description of the latter species is "Subgregaria, basi flavescenti-albida, apicem versus candida, dendroidea ramosissima, ramulis farinaceis. Ad elytra caraborum emortuum inter folia putrida in silvis prope Tjikoija, provinc. Batav. Mense Apr. Clavarioidea est parum fugax; stipes basi radiciformis subgeniculatis subsolidus; rami apice subfasciculati, fere crispi, in sinubus dilatati."

Hirsutella entomophila, in the available specimens, is brown, ashy at the tips, and therefore apparently differs from *Isaria ramosissima* in colour, though it is possible that it may be white at first. The other details of the latter species, as far as they go, do not agree with *Hirsutella entomophila*.

91. *Hirsutella formicarum* Petch, n.sp.

In *Trans. Brit. Mycol. Soc.* x (1924), 35, it was recorded that the conidial stage of *Cordyceps unilateralis* (Tul.) Sacc. occurred on the apex of the perithecial clava and was a *Hirsutella*. That has since been confirmed on specimens from British Guiana, collected by Mr Paul Richards, with the additional fact that the fungus may also produce independent conidial clavae.

In Mr Richards's specimens, the number of perithecial plates on a clava varies from one to four. If the tip of a clava has been broken off, a plate may bend over the broken end and simulate a terminal hemispherical head; or two plates may occur on opposite sides of the clava, with a similar effect. The perithecial clavae usually arise from the head of the insect and bear conidiophores towards the apex, but independent conidial clavae may arise anywhere, from the body, legs, or antennae. This conidial stage may be known as *Hirsutella formicarum*.

Hirsutella formicarum Petch, n.sp.—Clavis linearibus, usque 17 mm. alt., e corpore insecti oriundis, vel apicem stromatis Cordycipitis formantibus, brunneis, tomentosis, supra cinereis; basibus phialidum ovalibus, 8–11 × 4–6μ, in sterigma, 20μ alto, basi 1–1·5μ diam., sensim attenuatis; conidiis angusto-cymbiformibus, obtusis, 9–11 × 2μ, muco coalitis. Status conidiophorus *Ophiocordycipitis unilateralis*, British Guiana; Ceylon.

92. *Hirsutella radiata* Petch, n.sp.

Several examples of this species were collected by Dr C. B. Williams at Essequibo, British Guiana, September 11th, 1916. It occurred on a small fly, which was attached to a leaf by a small pad of brown mycelium.

The fungus arises from the thorax of the insect as a slender rigid column, 1–2 mm. high, 0·1 mm. diameter. From the apex of this basal column, rigid branches, up to 1 cm. long, 35–45 μ diameter, to the number of three or four, spread out horizontally, curving downwards at the tip. These branches may bear, near the distal end, a pair of opposite branches which similarly curve downwards. In some specimens, the basal column is continued upwards as a central stem for a length of about 7 mm., or this may curve over and appear as

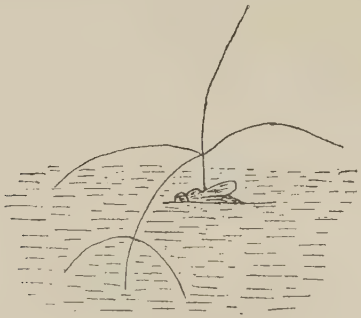


Fig. 4. *Hirsutella radiata.* ×6.

another branch. The colour of the whole fungus is dark brown or rufous brown, cinereous towards the tips, with a matt surface. The body of the host fly is only about 2 mm. long.

The phialides have a conical base, 5–8 × 3–4 μ, merging into a stout sterigma, 9–14 μ long, or a cylindrical base, 6–18 × 2 μ, with a sterigma 6 μ long. In many of the latter, the base and sterigma together form a uniformly conical whole and can be differentiated only by staining. The spore cluster is oval, 9–11 × 6–7 μ, and the individual conidia are cymbiform, 6–9 × 2–2·5 μ, or oval, 7–8 × 3–4 μ.

Hirsutella radiata Petch, n.sp.—Clavis erectis, rigidis, 1–2 mm. alt., 0·1 mm. diam., apice ramos horizontales ferentibus, truncatis, vel in flagellum usque 7 mm. alt. productis; ramis rigidis usque 1 cm. longis, 35–45 μ diam., apicem versus decurvis et saepe ramulos duos oppositos ferentibus; fusco-brunneis vel rufo-brunneis, apicibus cinereis; phialidibus basi conico, 5–8 × 3–4 μ, in sterigma, 9–14 μ

longum, attenuato, vel basi cylindraceo, 6–18 × 2 μ, sterigmate 6 μ longo; conidiis cymbiformibus, 6–9 × 2–2·5 μ, vel ovalibus, 7–8 × 3–4 μ.

On flies, British Guiana.

93. *Blastotrichum Aranearum* Petch, n.sp.

The genus *Blastotrichum* was established by Corda in *Icones Fungorum*, II (1838), 10, for a species, *Blastotrichum confervoides*, found on living and dead parts of a hydrophilous *Euphorbia*. From the submerged parts of the plant it spread through the water in lax woolly masses, but round the stem at water level it produced aerial tufts of crowded conidiophores.

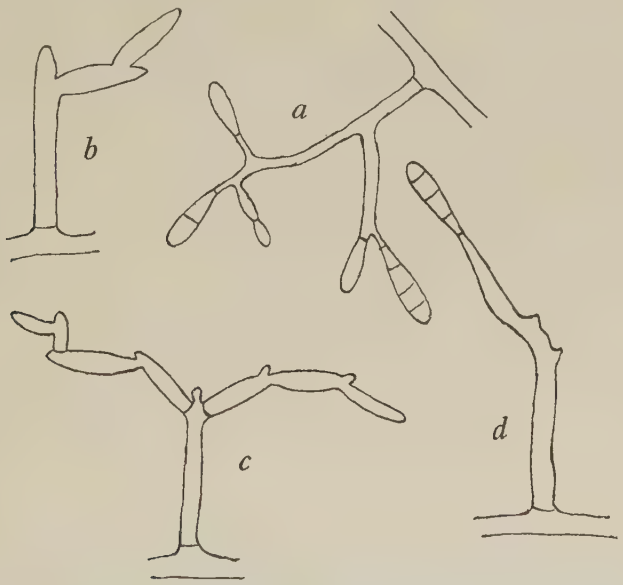


Fig. 5. *Blastotrichum Aranearum*. *a*, lax conidiophore from the body of the spider; *b*, *c*, *d*, rigid conidiophores from the legs. All × 1000.

A species referable to this genus has been found in Ceylon on spiders. In one specimen, in which the spider is concealed beneath a sheet of silk, the mycelium forms a loosely interwoven mat on the silk sheet, with small, waxy, pale amber, translucent masses of conidia; the conidiophores of this specimen are somewhat lax and decumbent, and have no septa, except the basal septum which cuts off the conidiophore from the hypha (Fig. 5*a*).

In another specimen, in which the spider was attached to a living leaf, without any cover, the conidiophores on the body of the insect are similar, but, in addition, conidiophores are present in rows along the legs of the spider, and these conidiophores are erect and rigid,

either sympodial (Figs. 5*b*, *d*) or cymosely branched. Some septa are present in the branched conidiophores, especially when the conidiophore bifurcates, a septum being then produced in each branch near its base (Fig. 5*c*).

Blastotrichum Aranearum Petch, n.sp.—Hyphis crassis, 2·5–3μ diam., insectum stromate laxo albo obducentibus; conidiophoris crassis, 2–3μ diam., intus granulosis, saepius non septatis, simplicibus vel cymose vel sympodialiter ramosis; conidiis hyalinis, fusoideis vel clavatis, utrinque rotundatis, 1–3 septatis, 16–24 × 3–3·5μ.

On spiders, Nuwara Eliya, Ceylon.

94. *Verticillium fuliginosum* Petch, n.sp.

This species was collected by Dr C. B. Williams on a leafhopper on sugar cane at Marienburg, Surinam, May 19th, 1916, and at Bocas, Panama, March 9th, 1917.

The mycelium on the insect is at first white, then fuliginous, and spreads from the insect over the leaf in a blackish film. The hyphae are stout, 5–10μ diameter, regular, pale fuscous, with black zones at the septa, which shade off on either side or sometimes extend over a whole segment. This peculiar coloration of the hyphae is very striking. The conidiophores are up to 500μ high, 7μ diameter at the base, attenuated upwards, septate, smooth, pale fuscous below, hyaline above, bearing one or two whorls of phialides towards the apex. The phialides are one-septate, the lower segment being cylindric or ovate, 18–20 × 7μ, and the upper segment flask-shaped or lanceolate or conical, 18–28 × 6–8μ, terminating in a slender sterigma up to 20μ long. The conidia are hyaline, broadly cymbiform, ends obtuse, 9–10 × 5–6μ, or oval, 7 × 5μ.

Verticillium fuliginosum Petch, n.sp.—Mycelio primo albo, dein fuliginoso, ab insecto folium in macula nigra tenui percurrente; hyphis crassis, 5–10μ diam. regularibus, pallide fuscis, septis nigerrimis; conidiophoris usque 500μ alt., infra 7μ diam. supra attenuatis, septatis, laevibus, infra pallide fuscis, supra hyalinis, phialides in verticillis singulis vel duobus apicem versus ferentibus; phialidibus uniseptatis, articulo infero cylindraceo vel ovato, 18–20 × 7μ, articulo superiori ampullaceo vel lanceolato vel conico, 18–28 × 6–8μ, in sterigmate tenui, usque 20μ longo abeunte; conidiis hyalinis, late cymbiformibus, utrinque obtusis, 9–10 × 5–6μ, vel ovalibus, 7 × 5μ.

On a leafhopper on sugar cane, Surinam and Panama.

95. *Sporotrichum columnare* Petch, n.sp.

In the Eastern tropics, *Cordyceps dipterigena* B. & Br. is parasitised by two species of *Sporotrichum*, viz. *S. album* Petch and *S. isarioides* Petch. In the Western tropics, another species of *Sporotrichum* has been found

parasitic on entomogenous fungi. This was first observed on a *Hirsutella* on flies, collected by Dr C. B. Williams at Essequibo, British Guiana, November 11th, 1916. Another specimen occurred on a spider cocoon, collected at Maricao, Porto Rico, March 23rd, 1916 (Cornell University, No. 730), and others on *H. entomophila* Pat., on beetles, Moruga, Trinidad, January 14th, 1921 (coll. W. Nowell), and on *H. floccosa* Speare, on froghoppers, Trinidad (E. M. Wakefield). In the spider cocoon, the host fungus was not identifiable.

In addition to a white film over the host fungus and insect, the *Sporotrichum* produces conical or cylindric clavae, scattered or clustered. The peripheral hyphae of these clavae terminate in simple conidiophores, usually geniculate towards the apex. The conidia, however, are not attached at the angles, as in *S. album*, but in a group just above an angle, and also at the apex of the conidiophore. Thus the conidiophore, in general, is geniculate, with minute papillae, the scars of attachment of the conidia, in small numbers just above each bend.

Fig. 6. *Sporotrichum columnare*. Conidiophore and conidium. × 1000.

As pointed out in *Trans. Brit. Mycol. Soc.* XVI (1931), 55, *Beauveria Peteloti* Vincens, from Brazil, is evidently *Hirsutella Saussurei* parasitised by a *Sporotrichum*. Vincens, however, figured the *Sporotrichum* as having geniculate conidiophores, with conidia broadly attached at the angles, as in *S. album*. Hence it seems improbable that the West Indian species is the same. I propose the name *Sporotrichum columnare* for the latter.

Sporotrichum columnare Petch, n.sp.—Mycelio insectum membrana alba obducente; clavis albis, sparsis vel congregatis, conicis vel cylindraceis, simplicibus vel furcatis, usque 1·8 mm. alt., 0·3 mm. diam., ex columna centrali strato laxo conidiophororum cincta compositis; conidiophoris simplicibus, 1·5–2 μ diam., flexuosis, supra geniculatis, angulis et apice minute verrucosis, apice clavato vel leniter inflato vel a latere flecto; conidiis hyalinis, angusto-ovalibus vel oblongo-ovalibus, uno fine acutis, 3·5–11 × 1·5 μ.

On *Hirsutella* spp., West Indies.

96. *Sporotrichum larvatum* Peck

In *Sylloge Fungorum*, x (1892), 533, Saccardo entered the description of *Sporotrichum larvatum* Peck, from the *Thirty-second Report of the New York State Museum*, p. 44, as follows:

"Caespitulis confluentibus, densis, mollibus, dein pulverulentis albidis vel flavidis, matricem totam obducentibus; hyphis tenuissimis, simplicibus vel ramosis; conidiis copiosis, minutis, globosis, 2–3μ diam. Hab. in larvis sub Alnis, Adirondack Mount. Amer. bor.—'An Botryti Bassianae affinis species?'"

The *Thirty-second Report* was never issued to the public. In the *Thirty-fourth Report* (for 1880, published 1881), the Director of the Museum commented upon the method of dealing with the scientific reports and stated (p. 5) that the scientific papers which accompanied the *Thirty-second Report* had not been published for the use of the Regents or for the Museum, but that the Report only had been printed among the State documents; and again (p. 11) "the *Thirty-second Report*, communicated in January, 1879, is printed simply as a public document, without the illustrations accompanying the scientific papers; no copies having been printed for the Regents or the Museum; and it is essentially inaccessible to the scientific public."

In the *Thirty-third Report* (for 1879, published 1880), Peck entered the name *Sporotrichum larvatum* Pk. in a list of plants new to the herbarium (p. 13).

In *Bulletin of the New York State Museum*, I, No. 2, p. 18 (May, 1887), Peck published a description of *Sporotrichum larvicolum* in a paper entitled "New Species of New York Fungi." The description is:

"Flocci slender, simple or branched, forming a continuous, dense, soft, white or yellowish stratum coating the whole matrix; spores abundant, minute, globose, 0·00008 to 0·00012 in. broad. Dead larvae lying on the ground under alders. Adirondack mountains. July. . . . In some specimens the fungus spores were so abundant that the surface of the stratum had a pulverulent appearance."

It will be seen that the descriptions of *Sporotrichum larvatum* and *S. larvicolum* are the same, differing merely in the order of the phrases, and the fungi are on the same host and from the same locality.

In the Bulletin in question, a note on the contents page states, "The titles of the first four articles were enumerated in the *Thirty-seventh Report on the State Museum*, but the articles were not printed. A revision of them is given here." On referring to the *Thirty-seventh Report*, it is found that Peck there stated, "I have also added to this part of the report descriptions of new species contained in the *Thirty-second Report*, but which were never published in such a way as to be generally available to the public, or to those most interested in having them"; and in the Table of Contents there is the title, "Descriptions of

New Species of New York Fungi." Finally, in the *Fortieth Annual Report* (published 1887), the Director of the Museum stated, "Since my report of last year was communicated to the Board of Regents, they have ordered an edition of the *Thirty-second Report* to be reprinted."

Thus Peck wrote his paper, "Descriptions of New Species of New York Fungi" for the *Thirty-second Report*, which was not issued to the public. He included it again in the *Thirty-seventh Report*, but it was omitted. It was finally published in the *Bulletin* in 1887, and the name *Sporotrichum larvatum* was there changed to *Sporotrichum larvicolum*. Confirmation of this is afforded by a comparison of the consecutive names in the paper in the *Bulletin* with those in the *Botanical Index to New York State Museum Reports* 22 to 38, included in the *Forty-first Report*. *Sporotrichum larvicolum* was the first effective publication.

Judging from the description, the fungus is *Beauveria Bassiana*. The dimensions given for the conidia are larger than is usual in that species, but I have examined a specimen of *B. Bassiana* from China, on a silkworm, in which the conidia were 2–4·5 μ diameter.

I am indebted to Mr E. W. Mason, of the Imperial Mycological Institute, for assistance in ascertaining the data concerning Peck's Reports.

97. *Oospora obducens* Syd.

This species was described by Sydow from the Philippines. It occurred on a Cicada, on which it formed effused, confluent, intense green masses. The conidia were narrow oval or oblong, obtuse, 9–13 × 3·5–5 μ, more rarely subglobose or broadly oval and 6–9 μ long. The specimen was said to be old and mycelium was lacking.

Except for the occurrence of some subglobose conidia, the details given agree with *Metarrhizium Anisopliae*.

98. *Metarrhizium brunneum* Petch, n.sp.

This fungus, on a Homopterous insect (Cicadellidae), collected at Laguna, Philippine Islands, December, 1931, was kindly sent me by Dr G. O. Ocfemia. It differs from *Metarrhizium Anisopliae* in colour and in the shape of its phialides, and from *M. album* in colour and the absence of a firm convoluted stroma.

Metarrhizium brunneum Petch, n.sp.—Insectum pulvere brunneo obducente; stromate basilari exiguo, ex hyphis laxe intertextis composito; phialidibus clavatis, usque 9 μ longis, supra usque 2–3 μ diametro incrassatis; conidiis cylindraceis vel anguste ovalibus, utrinque rotundatis, 4–6 × 1·5–2 μ, leviter flavis, coacervatis brunneis, catenulatis. Ad Homoptera (Cicadellidae), Philippine Islands.

The chains of conidia are laterally adherent in broad masses, which split into columns of spores, as in *M. Anisopliae*.

99. *Cladosporium*

In *Symbolae Mycologicae*, p. 356 (1869), Fuckel enumerated *Cladosporium herbarum* var. *Aphidis* Fuckel, without description. It occurred on dead aphids on *Cornus sanguinea* in Austria.

In 1877, Thuemen described a *Cladosporium* on aphids as *Cladosporium Aphidis*. This was included in Saccardo, *Sylloge Fungorum*, with a note that it might be the same as *Cladosporium herbarum* var. *Aphidis*, but it was certainly very different from *C. herbarum*.

In *Fungi Veneti*, v, 191, and *Mycotheca Veneta*, No. 587, Saccardo distributed specimens which he referred to *Cladosporium penicillioides* Preuss. These had been collected at Selva, Treviso, "in chrysalidibus ad folia adhuc pendula *Pruni domesticae*, Sept. 1875." In the copy of *Mycotheca Veneta*, No. 587, in Herb. British Museum, the fungus is on aphids, and it does not differ from that usually attributed to *Cladosporium Aphidis*. *C. penicillioides* was described by Preuss from specimens growing on *Tubercularia granulata* and *T. vulgaris*. It has been included among entomogenous fungi on the basis of Saccardo's record, and it should now be discarded.

In *Revue Mycologique*, 1890, p. 132, Briard and Hariot described *Cladosporium Aphidis* var. *Muscae*, found on a fly in France.

Giard in 1889 (*Bull. Sci. France et Belge*, xx, 217), instituted a new genus, *Polyrrhizium*, with the species, *P. Leptophyei*, for a fungus on an Orthopteron which he had previously referred tentatively to *Metarrhizium*. Giard was doubtful whether the fungus was not saprophytic, but decided that it was parasitic because the position of the dead insects was that assumed by insects attacked by parasitic fungi. A probable explanation of that is indicated below. There is little doubt, from Giard's figures and description, that the fungus was a *Cladosporium*. Giard himself stated that perhaps *Polyrrhizium* should be referred to *Cladosporium* or *Alternaria*, and that that would be another reason for considering it a saprophyte.

In 1891, Giard published a paper, entitled "Sur les Cladosporiées entomophytes, nouveau groupe de Champignons parasites des Insectes" (*Compt. Rend.* June 29th, 1891). This was a biological group, including, *inter alia*, Giard's genera, *Polyrrhizium* and *Penomyces*. He claimed that these fungi killed insects by blocking the tracheae.

In 1893, Rostrup (*Vidensk. Medd. naturh. Foren i Kjøbenhavn*, p. 95) remarked that Thuemen had described a *Cladosporium Aphidis*, which attacked aphids. Rostrup added that the occurrence was not uncommon, but the fungus was scarcely an independent species, confined to aphids, but a form of the ubiquitous *Cladosporium herbarum*, which had derived its nourishment from honeydew, whereby it grew very vigorously and spread over diseased or dead aphids.

Also in 1893, Giard (*Bull. Sci. France et Belge*, XXIV, 106) expressed the opinion that *Cladosporium Aphidis* Thuem. was only a variety of *C. herbarum*, viz. *C. herbarum* var. *Aphidis*.

Guéguen (*Champignons parasites*, 1904) recorded *Cladosporium herbarum* on a *Chermes* on *Mesembryanthemum edule*. According to Picard (*Ann. l'École Nat. d'Agric. Montpellier*, n.s., XIII (1914), 208), the name of the insect should be *Pulvinaria mesembryanthemi*. Guéguen suggested that the "Cladosporiées entomophytes," if not purely saprophytic, penetrated the insect only after it had been attacked by another parasite, Entomophthoraceae or perhaps bacteria.

In 1918, C. Massalongo described *Cladosporium herbarum* var. *aphidicola*, found on aphids on *Sonchus* in Italy. In *Sylloge Fungorum*, XXV (1931), 798, it was suggested that this is the same as *Cladosporium herbarum* var. *Aphidis* Fuckel, which was *nomen nudum*.

In 1923 (*Congrès Path. Veg. Strasbourg*, p. 48), Raybaud recorded, under the name *Cladosporium Lauri*, a fungus which had been found on the scale insects, *Aonidia lauri* and *Lecanium hesperidum*, on *Laurus nobilis*.

Giard's genus, *Penomyces*, was not entered in the *Sylloge Fungorum* until 1913 (vol. XXII). Giard did not give formal generic or specific descriptions, and it is scarcely possible to decide from his discursive accounts what he considered the essential characters of his genera. Guéguen (*Champignons parasites*) made an attempt to remedy Giard's omissions by summarising the facts given in his papers, but the data are really insufficient for precision, and one is still left wondering why Giard considered it necessary to create new genera.

In 1914 (*Ann. Myc.* XII, 295), Saccardo described, as *Penomyces cladosporiaceus*, a fungus found on mites on *Datura* in Moravia. Saccardo stated that *Penomyces* seemed to be distinguished from *Cladosporium* by having its conidia for the most part subglobose and continuous. Giard, however, did not describe the conidia of *Penomyces* as subglobose, and the dimensions which he gave for the species are not in accordance with that view. Saccardo's fungus would appear to differ from the common *Cladosporium* on insects, but it might still be included in that genus.

From the few details available, *Cladosporium parasiticum* Sorokin, found on a beetle, *Melolontha fullo*, in Russia, does not appear to be a *Cladosporium*.

Summarising the foregoing details, it is found that the Cladosporiums which occur on insects have been referred to the following species:

On aphids:

Cladosporium herbarum var. *Aphidis* Fuckel; also by Giard.
Cladosporium Aphidis Thuem.
Cladosporium herbarum Link, by Rostrup.

Cladosporium aphidicola Massalongo.
Cladosporium penicillioides Preuss; in error.
On scale insects:
Cladosporium herbarum Link; by Guéguen.
Cladosporium Lauri Raybaud.
On Orthoptera:
Polyrrhizium Leptophyei Giard.
On Coleoptera:
Penomyces telaria Giard.
Penomyces Cantharidum Giard.
Cladosporium parasiticum Sorokin.
On Hemiptera:
Penomyces telaria Giard.
On Diptera:
Cladosporium Aphidis var. *Muscae* Br. & Har.
On Acari:
Penomyces cladosporiaceus Sacc.

With the exception of *Cladosporium parasiticum* and *Penomyces cladosporiaceus*, these appear to be all the same species.

Rostrup stated that *Cladosporium herbarum* developed luxuriantly on honeydew, and spread thence to dead aphids. That the same *Cladosporium* grows both on honeydew and on aphids is true. I have observed this in aphids on beech leaves, on which the fungus grew on insect-free patches of honeydew and also on the aphids. But the fungus hyphae did not extend from the honeydew to the insects. The two growths were separate, though on the same leaf. Moreover, as a rule, the *Cladosporium* is not found growing on the leaf.

The *Cladosporium* which occurs on dead flies is undoubtedly the same morphologically as that on aphids, and on the former it usually follows an attack of *Empusa* or *Entomophthora*. It frequently happens that flies are killed by one of the Entomophthoraceae, but, probably because of a change in weather conditions, the insect dries up before the conidiophores of the fungus have emerged. Such individuals often bear *Cladosporium*.

That *Cladosporium* followed an attack of *Entomophthora* was suggested by Guéguen, who pointed out that that hypothesis would explain the facts noted by Giard in *Polyrrhizium Leptophyei* and *Penomyces Cantharidum*, viz. that the insects which Giard supposed were killed by these fungi died in the attitudes assumed by insects killed by Entomophthoraceae. It is almost impossible in such instances to determine the presence of the latter in the desiccated insect. In one instance, I found a dead fly attached to a leaf by its proboscis, after the manner of *Empusa Muscae*, but the only fungus recognisable was a minute tuft or nodule of *Cladosporium* at the tip of the proboscis.

As previously noted (*Trans. Brit. Mycol. Soc.* XVI (1932), 229),

Lecanium viride, in Ceylon, South India and Java, when attacked by *Empusa Fresenii*, is soon overrun by *Cladosporium* and other Dematiae, and it is difficult to find the *Empusa* on the insect. Similarly, in the Philippines, aphids attacked by the same *Empusa* have been quickly covered with *Cladosporium*. The growth of Dematiae in these cases is much more rapid than that on flies killed by Entomophthoraceae in Britain.

There is no doubt that *Cladosporium* will grow freely on insects killed by Entomophthoraceae, but it is open to question whether that will explain all occurrences of *Cladosporium* on insects.

Fuckel's name, *Cladosporium herbarum* var. *Aphidis* has been rejected as *nomen nudum*, as it was published without a description. Fuckel probably considered that the fungus did not differ from *C. herbarum*, except as regards its host.

100. A Sterile Stroma

A fungus which forms brown circular discs covering individual specimens of *Aspidiotus* was collected at Nuwara Eliya, Ceylon, on several occasions during 1926–8, principally on *Psychotria*, but in no instance was the fructification found.

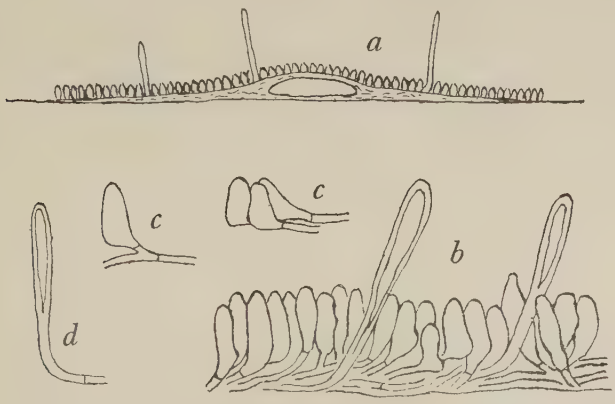


Fig. 7. A sterile stroma. *a*, cross-section (diagrammatic), ×40; *b*, part of radial cross-section, ×250; *c*, superficial cells, ×250; *d*, a seta, × 250.

The discs are circular, up to 3 mm. diameter, seal-brown with a paler fimbriate margin, becoming dark brown, minutely tomentose, flat, pulvinate in the centre, about 40μ thick, increasing to 150μ in the centre over the scale insect. They consist of a basal stratum of radial hyphae, which are brown, regular, 3μ diameter, to a thickness of about 10μ, but forming a thicker weft over and round the scale.

From this stratum arise shortly stalked, erect, brown cells, 20–30 μ high, 10–15 μ diameter, usually conoid with an obtuse apex, sometimes clavate and rounded above, closely packed side by side in a palisade layer, which, however, may be lacking over the scale. In addition, there also arise from the basal layer, dark brown, thick-walled setae, up to 200 μ high, 10 μ diameter, elongated clavate or cylindric, usually attenuated towards the base, darker than the palisade cells and sometimes almost sessile.

Some specimens bear small purple-red nodules. These are depauperate sporodochia of *Pseudomicrocera Henningsii*, growing on the scale insect, which have been partly overgrown by the brown fungus. No conidia, sporophores, or basidia of the brown fungus have been observed.

The fungus differs from a *Septobasidium* in that the stromata do not extend and coalesce. The setae suggest *Hymenochaete*, though they are not typical *Hymenochaete* setae, but no basidia have been found.

CORRECTIONS

The conidia of *Oospora subfasciculata* Petch (*Trans. Brit. Mycol. Soc.* xvi (1931), 63) are 2–3 × 1–1·5 μ, not 2–3 × 1·1–5 μ as originally printed.

The height of the perithecia of *Ophiocordyceps acicularis* is 0·48 mm., not 0·28 mm. as given in *op. cit.* xviii (1933), 60.

PORIA PEARSONII PILÁT SP.N.

By Dr ALBERT PILÁT
(*National Museum, Prague*)

(With Plate III)

In August 1933 and 1934, when collecting in Carpathian Ruthenia, I found in surprising abundance a *Poria* covering the prostrate trunks of *Abies alba* in a wood near the Kuzy brook at Velký Bočkov, a small town on the border of Czechoslovakia and Roumania, at a height of about 800 metres above the sea.

This *Poria* proved to be identical with that which Mr A. A. Pearson had doubtfully determined as *P. medulla-panis* Fries (non Persoon) and sent to Bourdot under that name (Bourdot in *Bull. Soc. mycol. Fr.* XLVIII (1932), 231).

This fungus in the dried state is coriaceous and more or less stratose, but when fresh is softer and saturated with water. It is a species that has been overlooked, though distributed throughout Europe and Northern Asia, especially in mountainous regions. Specimens have been gathered by Murashkinsky in Siberia and by Litschauer from two localities in Austria as mentioned in a letter to me.

Description of the Carpathian specimens

Poria Pearsonii Pilát sp.n.

Receptacle widely effused, confluent and often completely covering the prostrate trunks of trees, closely adherent but occasionally separating in places, when fresh white, watery-spongy, subfleshy then hard; in the dried state coriaceous, with the margin often slightly raised and subundulate. Subiculum of fine structure, membranous, white, irregular and penetrating into the fissures of the bark.

Tubes 2–5 mm. long, in most specimens more or less stratose in two to four layers; when fresh white, in dried specimens creamy or yellowish, brownish where rubbed.

Pores concolorous, angular-round, 0·12–0·40 mm. diameter or 0·50–1 mm. long.

Margin similar, often slightly raised in adult specimens.

Hyphae densely interwoven 2·5–3 μ thick, rarely 5–6 μ in subiculum more loosely compacted, horizontal.

Basidia 10–12 × 5–6 μ. Cystidia fairly numerous but not conspicuous, thick-walled 24 × 7 μ, projecting 5 to 10 μ, clavate-subfusoid, smooth or more often with apex incrusted with crystalline granules.

Spores not found in Siberian specimens, but in those gathered by A. A. Pearson in Somerset, England, on log of *Pinus sylvestris* October, 1929, and sent to Bourdot, the spores were widely elliptical or ovate, 3·5–4·5 × 2·5–2·75 μ: in Carpathian specimens, ovate, 3·7–4·2 × 26–27 μ.

Habitat. On prostrate trunks of *Abies alba* in Czechoslovakia—Carpathian Ruthenia, near the Kuzy brook above Velký-Bočkov July and August, 1933 and 1934, in great abundance.

On the bark of trunks of *Abies sibirica* in Siberia, district Kuznetzk, September 29th, 1930, collected by Krawtzew, and sent by Professor Murashkinsky.

We dedicate our species to Mr A. A. Pearson.

Latin diagnosis

Poria Pearsonii Pilát sp.n.

? *Poria medulla panis* Fries (non Persoon) comp. Bourdot, *Bull. Soc. mycol. Fr.* xlviii (1932), 231.

Exsiccata: A. Pilát: Fungi carpatici lignicoli, no. 129.

Carposomata late effusa, confluentia et totos truncos prostratos saepe obtegentia, sat firme accreta, solum in fragmentis *subseparabilia*, in statu vivo *alba*, *aquose-spongiosa*, *subcarnosa*, dein indurantia, exsiccata coriacea et marginibus saepe paulisper relevata et subundulata. Subiculum (trama) tenuissimum, membranaceum, album, irregulare, in rimas corticis penetrans.

Tubuli 2–5 mm. longi, in exemplaribus majoribus *plerumque stratosi* (bi-usque tetrastratosi), *albi*, exsiccati *cremei*, sublutescentes, in locis vulneratis saepe *subbrunnescentes*.

Pori concolores, angulato-rotundati, 0·12–0·4 mm. diam. vel 0·5 ad 1 mm. longi.

Margo similis, saepe in carposomatibus adultis paulisper relevatus.

Hyphae crasse tunicatae usque solidae, 2·5–3 μ crassae, rarius usque 5–6 μ, in subiculo sat laxe intricatae, horizontales, hyphae dissepimentorum subverticales, dense intricatae.

Basidia 10–12 × 5–6 μ. Cystidia *sat crebra*, sed haud conspecta, crassius tunicata, 24 × 7 μ ca. magna, 5–10 μ prominentia, *clavato-subfusoidea*, laevia vel saepius *capitulo crystallino* calcii oxalatici *coronata*.

Sporae in speciminibus Sibiricis haud observata. In speciminibus

carpaticis anno 1934 lectis sporas ovoideas, 3·7–4·2 × 2·6–2·7 μ magnas observavi. In speciminibus britannicis a cel. A. A. Pearson prope Somerset Britanniae ad ligna *Pini* Oct. 1929 lectis cel. Bourdot sporas late ellipsoideas vel ovoideas 3·5–4 (4·5) × 2·5–2·75 μ observavit.

Three large trunks, about 1 metre thick, of *Abies alba* were found by me entirely covered with the fungus. When fully developed, it is stratose, but the layers are never regular. It differs from *Coriolus obducens* (Pers.) in its appearance, the pores never acquiring when dry the attractive yellow tint found in *C. obducens*, which in its resupinate form is the same as *C. connatus*.

Some British mycologists, according to Bourdot (*loc. cit.*) have considered the fungus to be *Poria medulla-panis* sensu Fries non Persoon. The description, however, in Rea's *British Basidiomycetae* does not agree with our fungus and probably represents the true *P. medulla-panis* of Persoon. We also know that the specimen of *P. medulla-panis* in the herbarium of the Royal Botanic Gardens, Kew, appears to be the species of Persoon.

The *medulla-panis* of Fries in his *Systema mycologicum*, 1, 380, is thus described "effusus subundulatus, durus, glaber, siccus, albus, poris mediis. Ad ligna prostrata rarior."

This is a very brief diagnosis and corresponds more or less with both fungi. But Fries uses the word "durus" and this agrees more with the true *Poria medulla-panis* of Persoon than with our fungus which when fresh is fairly soft, spongy-watery and somewhat fleshy.

In *Mycologia Europaea*, II (1825), 100, the description agrees with the true *Poria medulla-panis*, and Persoon cites Fries in the synonymy.

A species very near to our fungus is *Poria subacida* Peck (Saccardo, *Syll. Fung.* VI (1888), 325. Syn. *Polyporus* (*Physisporus*) *subacidus* Peck in 38*th Rept. N.Y. State Mus.* pp. 92–3 (1885)) which is fairly common all over North America on coniferous wood. I have compared *Poria Pearsonii* with the accurate description and with the photographs of the original specimen of *P. subacida* which were published by Overholts in the *New York State Mus. Bull.* No. 205–6 (1919), pp. 111–15, t. 19, 20, 21, f. 6, together with the description and figures of specimens found by Shope in Colorado (Shope in *Ann. Missouri Bot. Garden*, XVIII (1931), 399, t. 39, f. 3), and also with the specimens in my private herbarium of this species on *Pinus virginiana*, mouth of Scott Run, Arl. W. Va. Coll. det. by J. R. Weir, November, 1922, and also on a log in a swamp at Vermihen, Mich., July, 1914, leg. A. Povah det. Overholts. *Poria subacida* Peck has a smoother surface of a darker yellowish brown. The hyphae in the dried specimens are always more or less yellow or yellow-brown, while in our species they are hyaline under the microscope. The American specimens of *P. subacida* which I have produce spores abundantly, while those of *P.*

Pearsonii are apt to be sterile; the stratification of adult specimens of *P. subacida* is also not so conspicuous and the sterile margin is more distinct; further in *P. Pearsonii* there is no marked marginal differentiation. In the texture of *P. subacida* I found quantities of crystals of calcium oxalate which Overholts and Shope mention, but the cystidia are indistinct and without incrustation. On the other hand *P. Pearsonii* has no crystals in the texture, but has cystidia with globose incrustations distinctly visible.

P. corticola Fries is nearly related, but certainly not the same (Pilát in *Bull. Soc. mycol. Fr.* XLVIII (1932), 38). This species grows on the bark of frondose trees, especially *Populus tremula*, and has characteristic cystidia.

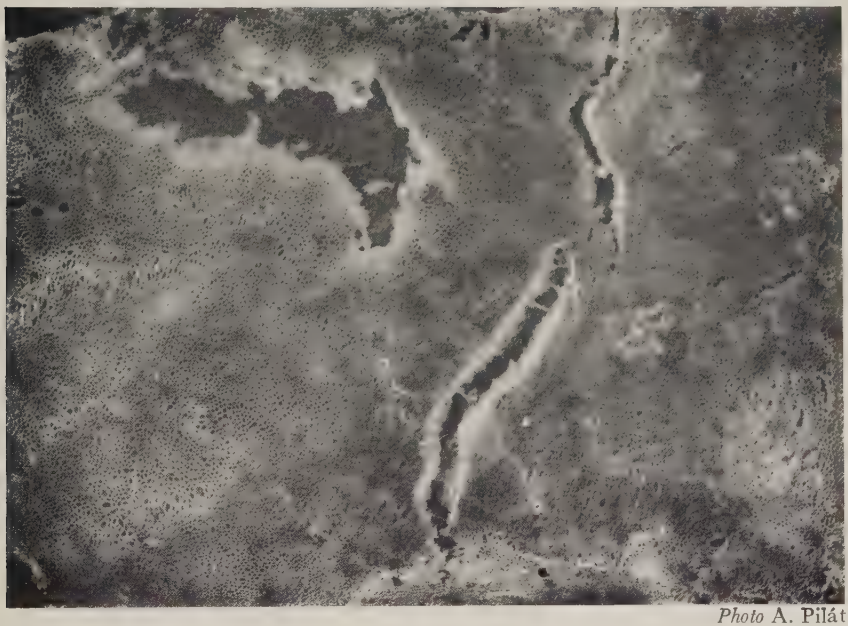
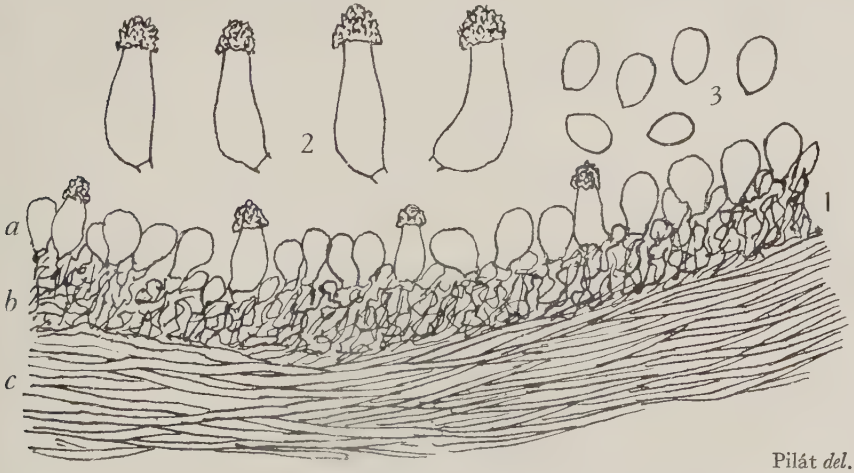


Photo A. Pilát

Fig. 1. *Poria Pearsonii* Pilát. Ad corticem truncorum prostratorum *Abietis albae* Mill. Čechoslovakia-Carpatorossia, ad rivum Kuzy supra Velký Bočkov, 1933.–VII.



Pilát *del.*

Fig. 2. *Poria Pearsonii.* 1, Hymenium in sectione: *a*, Basidia et cystidia; *b*, Subhymenium; *c*, Mediostratum. 2, Quatuor cystidia; 3, Sporae.

ON *LAMBERTELLA CORNI-MARIS* VON HÖHNEL, A BROWN-SPORED PARASITIC DISCOMYCETE

By T. H. HARRISON, D.Sc. and
A. F. EL-HELALY, B.Sc., Ph.D.

(With Plate IV and 4 Text-figures)

Introduction

In August, 1931, the senior author made a tour of some of the deciduous fruit areas of Western Europe with the object of studying the incidence of "Brown Rot" in fruits and of collecting material for critical studies of the organisms concerned. Search was made at all suitable points for apothecia arising from fruits mummified by the various organisms.

Some apothecia found on mummified apples in Switzerland, and others on mummified pears at two places in South Germany, 160 kilometres apart by road, appeared in the field to belong to *Sclerotinia fructigena* Ader. & Ruhl., a species for which apothecia have only once(1) been carefully described. Subsequent examination showed that the ascospores were brown, and further experience indicated that the fungus was *Lambertella Corni-maris* von Höhnel. This species was first found by von Höhnel, in 1917, on mummified fruits of the cornelian cherry (*Cornus Mas* L.) in Austria. A comparison of the material collected in 1931 with von Höhnel's type material showed that the samples were co-specific.

The fungus is of interest to plant pathologists, inasmuch as it causes a firm brown rot and mummification of several common fruits, and it has an exceptional tolerance of acidity. In pure culture on artificial media it readily forms apothecia in the laboratory, a point of interest to mycologists and teachers.

The senior author is responsible for the account of the occurrence, morphology and taxonomy of the organism; the junior author for the bulk of the cultural, physiological and pathological studies.

Occurrence and description of apothecia

The first batch of apothecia was found on mummified apples beneath a neglected apple tree on a hillside at Viège near Brügg, in the Upper Rhine Valley, Switzerland. The fruits on the tree were heavily infected with *Sclerotinia fructigena*. Mummified fruits lay in

abundance beneath the tree, some freshly formed, others at least a season old. Search in a moist, neglected spot on the lower side of the tree yielded apothecia, some on blackened mummified apples, some on unidentified fragments. The moisture and temperature of the locality were very similar to those which experience in Australia had shown to be ideal for the production of apothecia by *Sclerotinia fructicola*. It was considered at the time that these Swiss apothecia belonged to *Sclerotinia fructigena*, and it was felt that the opportunity of checking the description of this fungus by Aderhold and Ruhland should not be lost. As this was impossible at the time, material was sent to the Imperial Mycological Institute, Kew, where it was examined by Mr S. P. Wiltshire, whose notes are incorporated in this paper. He found that the ascospores were brown, and placed the fungus in the genus *Lambertella*.

Many of the apothecia were immature when collected, but Mr Wiltshire brought these to maturity, and induced others to develop, by keeping the mummified fruits in moist sand at 18–20° C. One of these fruits is shown on Pl. IV, fig. 1.

The fruit body was first visible as a minute sphere with the position of the disc discernible; it then elongated to a drum-stick-like object which expanded at the top to give the mature apothecium. The stipitate apothecia were at first crateriform, then saucer-shaped, and later flattened. Their colour varied with the location and age of the disc; some were pale pink to flesh coloured, others buffy citrine (Ridgway, Plate XVI), and others were dark brown. The disc ranged in diameter from 1 to 5 mm. The margin of the disc was uniform, obtuse and glabrous, except in young stages, when a few simple hyaline hairs were present. The apothecia were positively phototropic. The stipe varied considerably in length. Some apothecia were nearly sessile, others had a stipe 3 cm. long. The stipe arose from the blackened mummified fruit, and was black and very thin at the base, the extent to which these characters were present varying from specimen to specimen. The stipe became thicker and lighter in colour until it reached the bottom of the disc; this was lighter than the upper surface. The stipe was hairy, especially towards the base; rhizoids were absent.

For long distances in Switzerland and in South Germany the roads are lined with fruit trees, mainly apples and pears. Most of the trees are old and neglected. "Brown Rot", mostly caused by *Sclerotinia fructigena*, is prevalent in places and many mummified apples and pears can be found in warm, moist depressions beneath trees growing on sunny slopes, where dense herbage is usual. Apothecia were found on mummified pears from the previous season's crop in two situations of this sort, one by the side of the main road from Basle to Freiburg, the other on a grassy slope near the main road

from Freiburg to Baden-Baden. One pear, bearing twenty-one apothecia in various stages of development, was photographed *in situ* (Pl. IV, fig. 2). Another pear showing more clearly the details of stipe and disc is shown on Pl. IV, fig. 3.

The apothecia from the mummified pears were generally larger than those found on the apples in Switzerland. They were disciform to flat, flesh-pink to pale brown, and ranged in diameter from 1·5 to 7·5 mm. The stipe ranged in length from 0·5 to 20 mm., tapering gradually from the disc to the point of origin in the fruit; its colour graded from that of the disc to the jet-black of the substratum.

The details which follow apply equally to the apothecia from apples and pears, as it has been found that both belong to the same species.

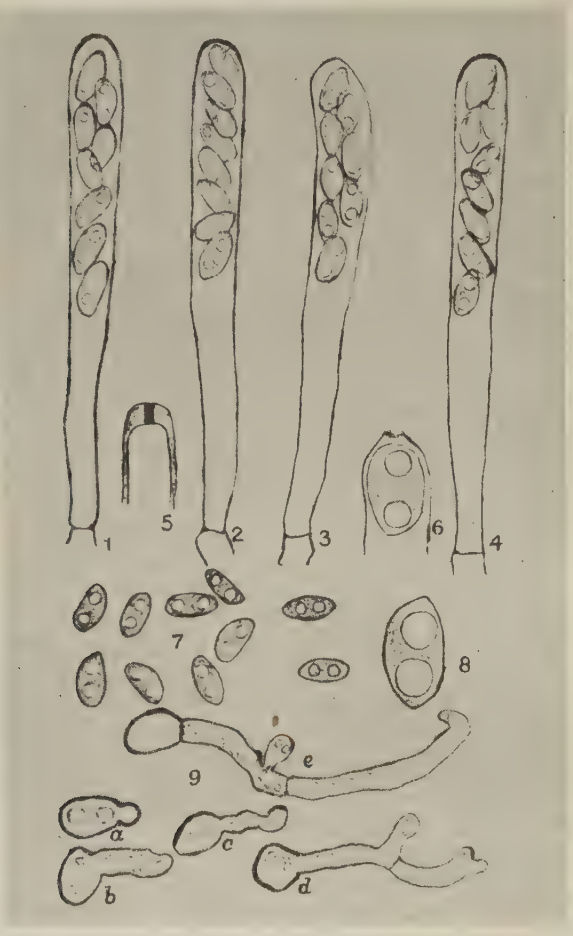
Asci. The asci are almost clavate and shortly stalked (Text-fig. 1, 1–4). The apex is somewhat flattened, with a centrally placed cylindrical pore which stains slightly with iodine. In young asci the apex is thickened, but this is much reduced at maturity (Text-fig. 1, 5–6). The asci measure 80–110 × 7–8 μ (widest diameter), tapering to 3–4 μ at the base. Average measurements are 100 × 7·5 μ.

Paraphyses. The paraphyses are colourless, sparsely septate, only slightly if at all swollen apically, usually unbranched, and projecting a little above the asci. They measure 100–120 × 1·4–3 μ.

Spores. The spores are irregularly ovoid, mostly somewhat flattened on one side, light brown in the centre, dark brown at the ends. Each spore contains two conspicuous rounded vacuoles, one towards either end of the spore (Text-fig. 1, 7–8). Occasionally the spores are colourless, but these are rare in mature asci. In young asci, the pigment appears to lie in the protoplasm of the ascus, the spores at that time being small, hyaline and very thin-walled. As the spores mature, the colour of the spore wall intensifies while the ascus becomes entirely hyaline. The spores measure 7·5–11·5 × 4–6·5 μ; the average of two hundred measurements was 9·8 × 5 μ. The spores are commonly monostichous, but just before discharge they become distichous. Care is required in mounting the preparation if the distichous arrangement is to be seen, as the slightest extra pressure causes discharge of the spores. It does not appear that the manner of arrangement of the spores in the ascus has any taxonomic significance in this type of fungus.

Germination of the ascospores. The ascospores germinate readily in distilled water and in many artificial media provided that the temperature is not too high for the fungus. The optimum temperature for growth and for spore germination is about 20° C. At this temperature, germination proceeds rapidly. Usually, a small swelling appears towards one end of the spore, the swelling enlarges, the spore assumes an angular outline and usually a single germ tube is produced. This

may become sinuous, or it may extend straight out for a considerable distance, usually branching laterally, and soon becoming septate (Text-fig. 1, 9 *a–e*).



Text-fig. 1. *Lambertella Corni-maris* von Höhnel. 1–4, Asci, showing arrangement of the spores. Camera lucida drawing from fresh material in distilled water, × 1000. 5, Thickened apex of young ascus, with central core which stains blue with iodine. The contents of the ascus at this stage are brown. 6, Apex of mature ascus, with a spore approaching the central pore; the wall thickening has disappeared. (5–6 drawn by Mr S. P. Wiltshire.) 7, Ascospores, × 1000. 8, A typical ascospore; one side somewhat flattened, and ends slightly pointed; vacuoles prominent. Camera lucida, × 2000. 9 *a–e*, Ascospores germinating in distilled water. Camera lucida, × 1000.

Cultural behaviour of the fungus

The organism grows readily on a large variety of media. On synthetic agar media such as Coons's, Brown's or Richards's and on the usual decoctions such as potato agar with or without dextrose, malt agar, etc., the fungus develops rapidly growing circular colonies which are practically devoid of aerial mycelium. On sterilised plugs of tissue such as potato, beetroot, carrot, apple or parsnip a powdery type of white aerial mycelium is shown.

While growth is free on a large variety of media, comparative tests showed that, judged by linear growth rate or by increase of dry weight of mycelium, the most favourable medium is one containing glucose and peptone as sources of carbon and nitrogen respectively. In the young stage the colony appears light yellow and the centre as a rule remains so throughout. Some little distance from the centre a darker zone arises after a time and this extends outwards with the progressive growth of the culture, the growing edge being still light coloured. The dark region becomes darker as the culture ages and in old cultures is almost black, especially on potato media.

After a time—about ten days in cultures incubated at 20° C. and longer at lower temperatures—thick dark brown pseudo-sclerotial crusts develop here and there over the surface of the culture. These are most prominent on potato and malt agars, the whole surface becoming more or less covered with a continuous thick skin.

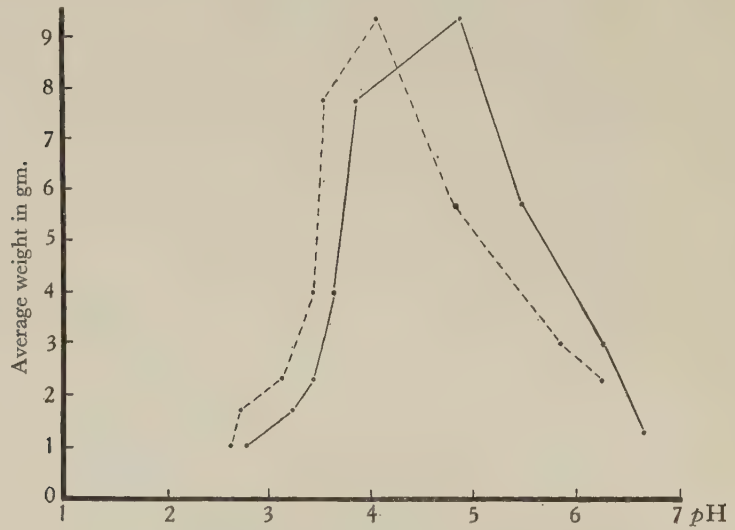
This crust gives rise later to the small spherical bodies which elongate to form immature apothecia: they are very similar to the female receptive bodies mentioned by Drayton (2), being possessed of numerous trichogyne-like hairs. Many become mature apothecia (Plate IV, fig. 4), the disc turning brown as the asci ripen. Sclerotia similar to those found on inoculated fruits have not been observed on artificial media. The sclerotial crust is not due to an accumulation of many small sclerotia, but is formed by the thickening of a dark brown surface incrustation. Ascospore clouds are readily obtained from apothecia growing in culture, and from such clouds it is possible to collect the spores on an inverted agar plate and to isolate the germinating spores. This provides a rapid and efficient method of obtaining single spore cultures.

The markedly acid-loving nature of this fungus is illustrated in Text-fig. 2, which gives the relation of initial *p*H of the cultural medium (Brown's solution) to the dry weight of mycelium produced. Whether the *p*H is determined electrically or colorimetrically, it is clear that the optimum for growth lies in the neighbourhood of *p*H 4–5.

Further experiments with agar media showed that the range of acidity over which growth takes place is *p*H 1·6–8·3, that the optimum for linear growth is at *p*H 4·4, and that vigorous growth occurs over

the range *p*H 3–7. In this respect *Lambertella* recalls the behaviour of the brown-rot fungi generally, as recently worked out by Hall (4).

The general tendency in the growth of this fungus is towards the development of greater acidity in the medium. When the initial reaction of the medium is on the alkaline side of the optimal point, the tendency during growth of the fungus is for the reaction to drift towards greater acidity. Thus on a starch-peptone medium adjusted to different acidities within the range *p*H 1·8–8·3, there was a drift of 0·5–1 unit towards the acid side in all the media of initial *p*H 5·9–8·3. Elsewhere the acidity remained unchanged.



Text-fig. 2. Illustrating effect of acidity upon mycelial growth of *Lambertella*.
—— Colorimetric method. ---- Electric method.

The general appearance of the culture varies according to the acidity of the medium. On starch-peptone agar medium with an acidity of 5·4 or less, the cultures present a clear glassy surface without trace of aerial mycelium and the medium is stained brown. With increasing acidity tufts of white aerial mycelium begin to appear, and over the range *p*H 4·4 to 2·4 the cultures present a white powdery appearance due to the development of abundant short aerial mycelium. The brown colour characteristic of less acid media has disappeared. At still higher acidities (*p*H 1·6–2·4) aerial mycelium again disappears on account of reduced growth.

The optimum temperature for growth was found to be near 20° C. The maximum temperature is unusually low, viz. 25° C. Only on Richards's solution agar was there any appreciable growth at the latter temperature.

Sporulation in culture

Oidium-like chains have been observed on certain media when the cultures have become rather old, *e.g.* in twenty days' old cultures on Richards's agar and one-month old cultures on Brown's A medium. On both media the cultures had become stale and showed a marginal fringe of short white aerial hyphae. In this marginal zone the oidium-like bodies were found in abundance. In no case, even after repeated examination, were they seen in young actively growing cultures.

These bodies appeared as typical oidia (Text-fig. 3) but were not seen to fall apart as separate spores, so that their significance is uncertain. Sometimes they appeared to be germinating *in situ*. From

Text-fig. 3. Monilioid chains (1–3) formed on hyphae of *Lambertella* in culture; 4, isolated conidioid body. Camera lucida drawing, × 1000.

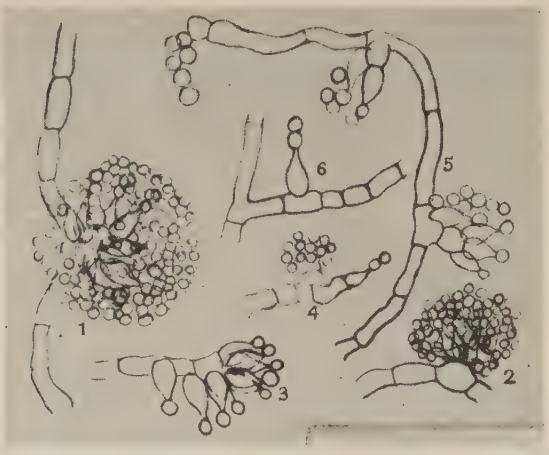
their mode of occurrence they may be looked upon as structures due to staling effects. They are very reminiscent of the similar structures seen in *Moniliopsis Aderholdi*.

Microconidia are at times produced abundantly in clumps as illustrated in Text-fig. 4. The heaviest production so far noticed has been on 2 per cent. plain agar, kept at 20° C. for seven days. They are produced in long fragile chains on flask-like structures. Usually the clumps are formed laterally on relatively thin, somewhat coiled, closely septate hyphae, but they may be terminal in origin. Most of the microconidia when abstricted are hyaline, thin-walled, spherical bodies approximately 3 μ in diameter. Germination of these bodies has not been observed.

Apothecia are freely produced on a variety of media under suitable conditions. The primordium of the fruit body appears as a minute dark brown outgrowth from the superficial crust of mycelium. This

elongates, reaching its full length in about five days, after which the apothecial cup develops, the latter process requiring about four days longer. The colour of the stipe remains unchanged throughout, viz. a pinkish buff at the top and clay colour at the base. The outer surface of the cup is clay coloured in both young and old stages, while the hymenial surface is clay coloured when young and sepia when old (Ridgway, Plate XXIX, 6). In length of stipe and diameter of cup the artificially produced apothecia agreed well with the natural ones already described.

Apothecia are formed only when the culture reaches a certain age of development and are confined to the thick dark brown or black



Text-fig. 4. Microconidia of *Lambertella* produced in culture. Camera lucida drawing.

pseudosclerotial crusts which develop over the surface of the culture. They appear after about seven weeks from the date of inoculation.

The average size of asci produced in culture was $110 \times 8{\cdot}7\,\mu$, *i.e.* they are somewhat larger than those naturally occurring. The size and appearance of the ascospores were essentially the same in both.

Light appears to be a factor of considerable importance in stimulating the formation of apothecia. This was suggested by the behaviour of the cultures during the first year after isolation. The mummies were collected in the autumn of 1931, and by the time artificial cultures were prepared it was already November. Throughout the winter cultures were set up on a variety of media at different times and exposed to diffused light on the laboratory bench near a window. No apothecia appeared, but from time to time an occasional stipe. In the following spring and summer, fully developed apothecia

appeared in abundance under the same treatment. The following experiment illustrates the effect of light more critically.

Cultures were set up on 2 per cent. malt agar in plates and tubes. Some of these were wrapped in black paper, others in white paper and the remainder in transparent (cellophane) paper and placed near a window. The appearance presented after about seven weeks was:

Cultures wrapped in cellophane, numerous mature apothecia.
„ „ white paper, numerous long stipes.
„ „ black paper, no fruiting structures.

This experiment was carried out three times with the same result.

A high degree of atmospheric humidity is necessary for formation of apothecia. This was shown by the following experiment.

Eight plate cultures on 2 per cent. malt agar were prepared, and after incubation for three weeks in the ordinary way at laboratory temperature were placed, after removal of the lids, as follows: four in a large container kept moist with water and four in a similar vessel containing a strong solution of calcium chloride. Only the former developed apothecia.

During the summer of 1933 it was noticed that when the temperature rose above 25° C. apothecia no longer developed, while those which were already present rapidly withered and disintegrated. Once the temperature again fell below 25° C. they began to appear again.

The composition of the medium is of some importance. In a comparative test of various media, apothecia were formed on the following:

Potato dextrose agar	200 gm.	potato per litre	
	15 „	dextrose	„
Malt agar	20 „	malt	„
Prune agar	20 „	prunes	„
Coons's agar			
Oatmeal agar	50 „	oatmeal	„

these media being arranged in descending order of suitability. On potato extract agar (200 gm. potato per litre) and on Brown's A and Richards's agar no apothecia were formed. Apparently the development of apothecia is dependent upon the formation of pseudosclerotial crusts, and these do not appear on the media last mentioned.

The effect of acidity was tested by preparing starch peptone media with initial *p*H as follows: 2·2, 2·6, 3·0, 4·0, 4·4, 5·0, 5·4, 6·2 and 6·8. It was found that stipes first appeared in the culture of *p*H 4·4, then later at *p*H 4·0, and later still at *p*H 3·0, 5·0 and 5·4. So far no stipes have appeared outside the range *p*H 3·0–5·4.

Secretion of enzymes

1. *Oxidising enzymes*

In a previous section the tendency of *Lambertella* to produce a brown to black discoloration of the medium, especially on natural decoctions, has been noted. Later it will be shown that similar effects are produced on parasitised fruits. This behaviour suggested that oxidising enzymes might play a part, and for that reason the following determinations were carried out.

The two reagents used were guaiacum emulsion and pyrogallic acid prepared according to the recommendations of Wormald(11), as follows: To 10 gm. of gum guaiacum were added 100 c.c. of 95 per cent. alcohol, the mixture shaken at intervals over three days, and then filtered. The red-coloured filtrate was used as stock solution. The emulsion was prepared freshly for each test by shaking 5 c.c. of the tincture with 95 c.c. of distilled water, a white emulsion resulting. In carrying out the test, 1 c.c. of the liquid to be examined was added to 5 c.c. of the emulsion in a test tube. This method can be used to a certain extent quantitatively by noting the time required for the appearance of a blue colour of standard intensity.

The pyrogallic acid reagent was prepared freshly for each test, a 2 per cent. aqueous solution being used. In carrying out the tests 2 c.c. portions of the medium under examination were pipetted into test tubes and 5 c.c. of the acid added to each. A yellow coloration appeared in the presence of oxidising enzymes.

Controls were used throughout the tests by placing the tubes containing the culture solution in boiling water for twenty minutes before adding the reagent.

For comparative purposes cultures of *Lambertella*, *Monilia fructigena* and *Sclerotinia aestivalis* were tested in this respect. Each was grown in small flasks containing 30 c.c. of apple extract and incubated at 20° C. It was noted that after two days' growth *Lambertella* had produced a dark colour in the medium and that *Monilia* gave the same degree of darkening only after five days. On the other hand *S. aestivalis* gave no colour change. When the cultures were seven days' old, the liquid of each was filtered off and tested with guaiacum emulsion.

Table I gives the times required for certain standard colours to develop in the various tests.

From both sets of results it is clearly indicated that *Lambertella* is more active in excreting oxidising enzymes than is *M. fructigena* and that again the latter is more active than *S. aestivalis*. As the development of colour on media follows the same order, there are grounds for suggesting that oxidising enzymes are the active agents concerned.

Table I.

Fungus	Pale blue*	Caerulean blue*
Lambertella	10 mins.	45 mins.
M. fructigena	45 ,,	80 ,,
S. aestivalis	120 ,,	148 ,,
Fungus	Beryl blue*	Light methyl blue*
Lambertella	10 mins.	45 mins.
M. fructigena	45 ,,	115 ,,
S. aestivalis	115 ,,	115 ,,

* As in Ridgway.

2. *Pectinase*

The capacity of *Lambertella* to attack apple fruits, to which reference will be made later, suggested that it is able to excrete the cell-wall dissolving enzyme, pectinase. Tests were carried out, and for purposes of comparison *Monilia fructigena* and *Sclerotinia aestivalis* were also examined. It should be noted that the parasitic vigour of these three fungi diminishes in the order *Monilia*, *Lambertella*, *Sclerotinia*.

Cultures were set up in tubes on sterilised turnip and apple plugs. After a certain time the juice was extracted and its activity tested on potato discs of standard thickness. The time required for loss of coherence of these discs to take place is in inverse ratio to the activity of the extracts. Also controls were set up in which boiled extracts were used.

In no case was the presence of enzyme detected in cultures of *S. aestivalis*. Table II gives the times required for decomposition of the standard discs in various extracts from turnip-plug cultures of *Lambertella* and *Monilia*.

Table II.

Age of culture	*Lambertella*	*Monilia fructigena*
10 days	570 mins.	120 mins.
17 ,,	225 ,,	40 ,,
24 ,,	170 ,,	35 ,,
31 ,,	90 ,,	30 ,,
38 ,,	90 ,,	30 ,,

Similar results were obtained with cultures on apple plugs. The controls were inactive in all cases.

Cultures of all three fungi likewise gave positive tests for the enzymes diastase, invertase and maltase.

Pathogenicity

In carrying out inoculations of fruits, Granger and Horne's (3) method as somewhat improved by Vasudeva (10) was used. The most extensive tests were made with apple fruit, and in this connection a comparison was made of the rate of invasion by the three fungi,

Lambertella Corni-maris, *Monilia fructigena* and *Sclerotinia aestivalis*. To control the error due to variable resistance of different apples, both fungi in any comparison were inoculated into opposite sides of the same apple. Uninoculated but wounded controls were set up as usual, and reisolations from infected fruits were carried out.

Table III gives the average surface spread and weight of rotted tissue in a batch of four apples inoculated with *Lambertella* and *S. aestivalis* after ten days' incubation at 20° C.

Table III.

	Lambertella	*S. aestivalis*
Diameter of lesion (cm.)	4·5	2·7
Weight of rot (gm.)	15·2	3·0

Table IV gives a similar comparison of *Monilia fructigena* and *Lambertella* over a range of temperatures.

Table IV.

	10° C.		15° C.		20° C.	
	M.	*L.*	*M.*	*L.*	*M.*	*L.*
Diameter after 6 days (cm.)	3·2	1·8	4·0	2·2	6·5	2·3
Weight of rot ,, ,, (gm.)	9·0	2·1	23·4	3·9	52·5	2·9

Tables III and IV show clearly that *Monilia fructigena* is a more active parasite of apple fruit than is *Lambertella*, which again is more active than *Sclerotinia aestivalis*.

Further inoculations showed that *Lambertella* attacks pears and plums both ripe and unripe, orange, lemon (Plate IV, fig. 5), quince, and such vegetables as turnip and parsnip. Potato and carrot were not attached. Invasion of orange and lemon took place chiefly along the locular walls. Artificially inoculated plums (Plate IV, fig. 6), after being kept a few months, were shrunken and rubbery in texture. Brown at first, they finally become covered with an almost continuous layer of black pseudosclerotial crust-like material.

A firm brown rot is caused, especially in apples and pears (Plate IV, figs. 7 and 8). The rot gradually extends throughout the flesh, thoroughly absorbing it and forming a pseudosclerotium with the toughness and elasticity characteristic of the brown rot fungi, *Sclerotinia fructigena* and *S. fructicola*. These species, however, give a black pseudosclerotium, whereas that of *Lambertella* is brown. On the surfaces of fruits thus mummified, there may appear small white masses of hyphae, spherical to dome-shaped and closely resembling the immature pustules of *S. fructigena* on apple. (Plate IV, fig. 8, right). No spores are produced. The hyphal mass thickens and gradually darkens until dark sclerotioid bodies are scattered all over the surface of the mummifying fruit. These possess the power of

"creeping"—the fungus grows out from the edges of these bodies, forming a thin crust over much of the surface of the fruit. No conidial stage has been seen on infected fruits.

Inoculations of plum and apple blossoms in the spring of 1933 indicated that attack had taken place inasmuch as none of the inoculated flowers subsequently set fruit. Here the inoculum of mycelium with malt agar was laid inside the flower. Flowers in which malt agar only was used set fruit in the normal way. Unfortunately intermediate stages were not observed, so that while there is reason for thinking that the fungus attacked the blossom the experiment stands in need of confirmation.

A parallel series of wound inoculations into branches of apple, pear, cherry and plum gave negative results; all the wounds become callused over in due course. This was true both for inoculations of the current and the previous year's wood.

Taxonomic discussion

The present record appears to be the only one since von Höhnel described apothecia received from Lambert in Austria in 1917. Von Höhnel's collection is preserved in the Farlow Herbarium at Harvard and contains twenty-nine mummified fruits, one of which bears eleven apothecia. Examination of some of this material showed that it agreed in all points of taxonomic value with the fungus collected on apples and pears in 1931. From the accompanying notes it is clear than von Höhnel noted the change in colour of the spores from transparent when young to dark brown when mature. He gives a diagram of hyaline spores with rounded ends, measuring $8 \times 4\,\mu$, and another of dark spores with more pointed ends, measuring $8–12 \times 4\,\mu$. In another note the measurement of the asci is given as $80 \times 6–8\,\mu$, but generally $100 \times 8\,\mu$ when they are mature. The full description (*loc. cit.*) is unaccompanied by figures.

Von Höhnel's record is listed by Strasser (9) and by Saccardo (8). The latter states that *Lambertella* "est *Sclerotinia phaeospora*"; von Höhnel describes his fungus as a "*Stromatinia* mit gefärbten Sporen".

There are however two other records of a *Sclerotinia* with brown spores. In 1912 Sasaki discovered apothecia with brown spores arising from mummified apples in Japan. The fungus was described by Hori (6), who regarded the brown spores as sufficient justification for a new genus, *Phaeosclerotinia*, and named the fungus *P. nipponica*. Later (7) he apparently revised his opinion and altered the name to *Sclerotinia phaeospora*.

Hori examined *P. nipponica* in culture and showed that it had a *Monilia* stage. It was probably on this account that he subsequently transferred it to *Sclerotinia* as *S. phaeospora*. Von Höhnel does not

mention a *Monilia* stage, nor has such been found in the present case, even after extensive search. The Japanese fungus may thus prove to be distinct from that of von Höhnel. The point can be determined only by a careful comparison of the descriptions and material. It will be noted that, if the two fungi are identical, and if the name *Phaeosclerotinia nipponica* was properly published before 1918, then *Lambertella Corni-maris* von Höhnel becomes a synonym of *Sclerotinia phaeospora* Hori. Incidentally, the specific epithet *Corni-maris* is not a very happy one for a fungus which possesses a much wider range of hosts than the name indicates.

The second occurrence of a *Sclerotinia*-type of fungus with brown ascospores is in a collection of Bermudan fungi made by Prof. H. H. Whetzel in 1923–4 and now preserved in the Plant Pathological Herbarium at Cornell University. This material has not been fully described and so far is provisionally labelled as *Sclerotinia* sp.

According to Whetzel* *Lambertella Corni-maris* is a typical *Ciboria*, both in its general characteristics and its cultural behaviour. He considers that, even among the Discomycetes, the colour of the ascospores alone is not sufficient to justify generic rank. On the other hand, von Höhnel evidently thought that spore colour was important. Variation in spore colour within a genus is common among the Pyrenomycetes, but is so rare in the Discomycetes as to constitute a striking feature. Certainly the general view of mycologists in England with whom the matter was discussed was that von Höhnel was justified in proposing his new genus.

Prof. Whetzel's suggestion that *Lambertella Corni-maris* is a species of *Ciboria* is not at present acceptable. The taxonomy and nomenclature of the Sclerotiniae is in process of revision, and it is hoped that before long Prof. Whetzel will publish characters indicating at least the generic concepts held. The line of demarcation between *Ciboria* and *Sclerotinia* is difficult to determine by consulting the existing definitions and descriptions of these overloaded genera.

Finally, Dr E. E. Honey* in an unpublished monograph proposes to include the Japanese fungus under the genus *Monilinia* on the ground that the colour of the ascospore does not prevent the fungus from being a good representative of the "Monilioid Sclerotinias." The status of *Monilinia* would come up for consideration in the present connection if it were shown that *Lambertella* possessed a *Monilia* stage.

In conclusion, while the authors agree that the validity of von Höhnel's genus *Lambertella* is somewhat in doubt, they suggest that the name *Lambertella Corni-maris* von Höhnel be retained until a strict examination of all the relevant material has been made.

* Private communication.

The authors are indebted to the following gentlemen for facilities in their various institutions: Dr E. J. Butler and Mr S. P. Wiltshire of the Imperial Mycological Institute, London; Mr J. Ramsbottom, Department of Botany, British Museum (Nat. Hist.); Prof. W. H. Weston, Botanical Department, and Mrs Riddle, Farlow Herbarium, Harvard University; Prof. H. H. Whetzel, Cornell University; and finally Prof. W. Brown, Imperial College of Science and Technology, London, in whose laboratory all the cultural work was carried out.

Summary

1. A Discomycete with brown ascospores was collected from mummified apples in Switzerland and from mummified pears in Germany in August 1931.

2. Taxonomic details are presented to show that it is identical with one collected from fruits of *Cornus mas* in Austria in 1917.

3. The fungus grows on a large variety of media. A thick dark brown or black pseudosclerotial crust develops over the surface of the culture on most of the media. The optimum *p*H for growth is at a point of relatively high acidity, viz. near *p*H 4·4; and growth occurs over a wide range of *p*H, viz. from 1·6 to 8·3.

4. The existence of an imperfect stage is doubtful, whereas apothecia with ascospores are produced on a variety of media under suitable conditions of light, humidity, temperature and acidity. They are confined to the black pseudosclerotial crusts.

5. The fungus is very active in excreting oxidising enzymes as well as pectinase.

6. Under laboratory conditions the fungus attacks a variety of fruits and vegetables (apple, pear, plum, quince, orange, lemon, turnip and parsnip). Inoculations of apple and plum blossoms appeared to give positive results, whereas no attack resulted in inoculations of young wood of apple, pear, cherry and plum.

7. The nomenclature of the fungus is discussed and the conclusion reached that for the present the name for the organism must be *Lambertella Corni-maris* von Höhnel.

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EXPLANATION OF PLATE IV

Fig. 1. Apothecia from mummified apple found at Viège, Switzerland. ×1·8 approx. Phot. S. P. Wiltshire.
Fig. 2. Mummified pear bearing 21 apothecia. Photographed *in situ*, Baden-Baden, August 1931. Nat. size.
Fig. 3. Detailed view of apothecia on mummified pear. Nat. size.
Fig. 4. Apothecia produced on 2 per cent. malt agar culture kept in daylight. ×0·8 approx.
Fig. 5. Infected orange (l.) and lemon (r.).
Fig. 6. Branch of plum bearing infected fruits.
Fig. 7. Inoculated pears, after ten days at 20° C.
Fig. 8. Inoculated apples.

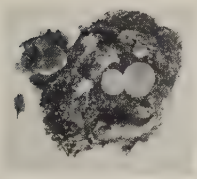

Fig. 1

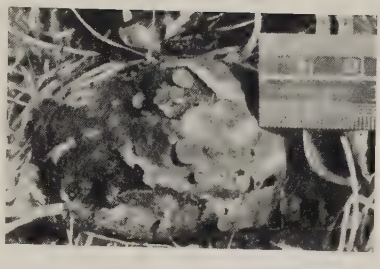

Fig. 2

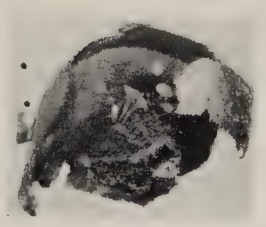

Fig. 3

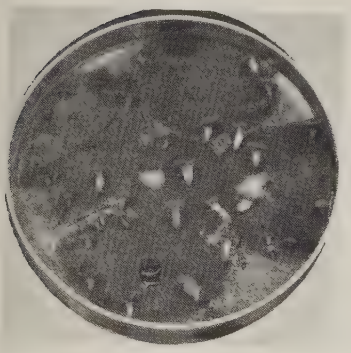

Fig. 4

Fig. 5

Fig. 6

Fig. 7

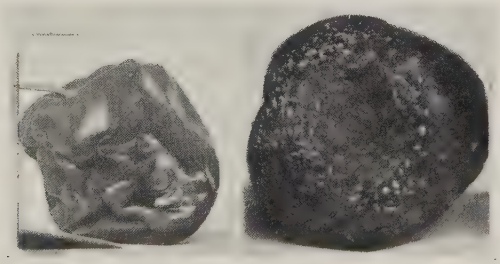

Fig. 8

NEUROSPORA IN BRITAIN

By J. RAMSBOTTOM and F. L. STEPHENS

(With Plates V–VII)

"*Monilia sitophila* (Mont.) Sacc." as a collective species has become one of the best known and most important fungi owing to the work of Shear and Dodge (1927) and Dodge and his pupils since that date. Their discovery of the perfect stage for which they instituted a new genus, *Neurospora*, has had considerable influence on mycological thought. The purpose of the present paper is to record two species of *Neurospora* for this country but it seems advisable to note certain points which were studied in making the identifications.

The fungus is called "red bread mold" by Shear and Dodge because of its frequency as a bakery pest. In 1842 the French Minister of War appointed a commission to investigate the cause of an infestation of Army bakeries. Léveillé (1843) named the fungus *Oidium aurantiacum* and the same year Montagne called it *Penicillium sitophilum*; it was transferred to *Monilia* by Saccardo (1881). Outbreaks of the fungus have also been recorded from Germany, Italy, and U.S.A.; we know of no such trouble in the British Isles.*

Petch (1931) has given an account of the recorded occurrences of *Monilia sitophila* in this country. These are:

On rotting seed after the burning down of an oil mill, King's Lynn, 1899. C. B. Plowright.

On charred grain after a mill-fire, Grantchester, 1928–9. F. T. Brooks.

On burnt trees, Bawsey, near King's Lynn, 1929. T. Petch.

Petch says "It is curious that there are no previous records of this fungus in Great Britain. One would have expected that it would have occurred on charred gorse after heathfires." One of our specimens is from such a habitat. It was found while demonstrating the conidial stages of *Daldinia concentrica* at a fungus foray of the Plumstead and Woolwich Natural History Society at Woolwich, on October 2nd, 1933. There had been a fire during the summer and

* We have since learned from the Plant Pathological Laboratory, Harpenden, that the fungus occurred in a bakery in the Irish Free State in August, 1933. Dr E. A. Fisher informed Dr G. H. Pethybridge that "he believes that outbreaks of this fungus in bakeries in England are rather uncommon." Mr C. G. C. Chesters has kindly sent us cultures which he isolated from dough sent to him from a bakehouse "several years ago": the material contained ascospores.

the scorched stems of *Ulex* showed a salmon-pink growth which was obviously not *Pyronema confluens*. It is not unlikely that the fungus is fairly common in such conditions, but so far we have no confirmation of this belief.

We have also obtained a culture from the National Collection of Type Cultures. It had been sent to the Lister Institute by Mr G. Smith who informs us that he obtained it about three years ago when he was plating out hundreds of samples of cotton yarns. "It occurred occasionally as a single colony on one plate of a series and was most probably just a chance infection."

While the first fungus was being investigated Mr W. H. Wilkins found a second form growing in enormous quantity on the ends of beech battens which were being kiln dried in a timber yard at Chichester.

The apparent rarity of the fungus is surprising when its extensive distribution in America is considered, for there the fungus (*sens. lat.*) infects sugar-cane bagasse and silage and causes a storage rot of fruits especially strawberries, raspberries and apples. It is also "well-known to laboratory workers in mycology and pathology as a contamination in cultures of other organisms."

There is no doubt that the fungus found commonly on charred material in the tropics and usually called *Monilia carbonaria* Cooke belongs to the same group of species; Cooke's species was described from burnt wood and stems in New Zealand. "People's utmost attention was directed towards a remarkable reddish yellow fungus which has grown up luxuriantly on the bark of burnt or half-burnt trees after a few days of the terrible fire of Tokyo on September 1, 1923. The fungus was found to belong always to the same species, though a number of Coniferous as well as broad-leaved trees were over-grown with it. It appears externally just like some wild yeast which very commonly is found living on the exudation of some trees or on some organic substances, but under the microscope it reveals itself evidently to be a certain species of *Monilia*" (Kitazima, 1925).

Shear and Dodge give the synonomy of *Monilia sitophila* (Mont.) Sacc. as: *Penicillium sitophilum* Mont.; *Oidium aurantiacum* Lév.; *Monilia Martii* Ell. & Sacc.; *Oospora aurantiaca* (Lév.) Herter; *Monilia aurantiaca* (Lév.) Herter non Peck and Saccardo; *Oidium Lupuli* Matth. & Lott.; *Oospora Lupuli* (Matth. & Lott) Lindau; *Monilia aurea* Kitazima non Gmelin; and they query *Monilia carbonaria* Cooke.

The inclusion of *Oidium Lupuli** is noteworthy because it was first described from spent hops in this country. The original account reads: "*Oidium Lupuli* is an excellent example of a mould resembling *Oidium lactis* in its mode of growth; it is occasionally met with on spent hops,

* *Monilia Lupuli* Mass. apud Grove in *J. Econ. Biol.* VI (1911), 412.

on which it forms a reddish yellow or salmon-coloured dust, which on microscopical examination, is found to consist of branching cells, merging like *Mucor Racemosus* into spherical cells, some of which have all the appearance of budding. Many of the spherical cells and the branching pieces display an orange-pink colour, which seems to permeate the protoplasm. For a purely aerobian form, the mode of reproduction is decidedly interesting" (1889, pp. 86–7). This is the same fungus as *Oidium aurantium* Cooke issued in *Fungi Britannica Exsiccati* No. 448 and also in Rabenhorst's *Fungi Exs.* No. 1579 and described in *Grevillea*, I (1872), 21: "*Oidium aurantium* Cooke. 'Orange oidium.' Forming dense, irregular, effused, bright orange tufts, sometimes several inches in length. Hyphasma creeping, branched, robust, septate, surmounted by simple or branched moniliform threads, which break up into subglobose or elliptical spores; endochrome granular.—Cooke exs. No. 488, on spent hops. Burton-on Trent. August 1871 (Edwin Brown, Esq.)."

This species appears in Saccardo, *Sylloge Fungorum*, IV (1886), 22 as *Oospora aurantia* (Cke.) Sacc. & Vogl (cf. Massee, *Fungus Flora*, III (1893), 280).

According to information we have received this fungus is not infrequent in this country. It was noticed on hop manure exhibited at the Chelsea Flower Show in 1930* and has been kept in culture since. Shear and Dodge are correct in assigning it to *Monilia sitophila*†.

The fungus which was gathered on burnt *Ulex* was cultured. After a few days perithecia formed and on examination were found to agree exactly with the description of *Neurospora tetrasperma* Shear & Dodge. This was a matter of great surprise for this fungus appears to have been known previously only from Surinam, Dutch Guiana, "and a few places as yet" (Dodge, 1930).

Neurospora Shear & Dodge in *J. agric. Res.* XXXIV (1927), 1025

"Perithecia gregarious or scattered, smooth or with loose hairs, subcoriaceous to subcarbonous; ostiole papillate or short rostrate, perithecial cavity lysigenic, filled at first with parallel, septate hyphae (paraphyses?) which begin to collapse and disappear as soon as the young asci start and are entirely gone when the asci are full grown; spores hyaline to yellowish-brown at first, becoming black or greenish black when mature, continuous, longitudinally ribbed. Conidial stage of the *Monilia sitophila* type.

The perithecia are either superficial or embedded when grown on agar media; when superficial they usually show a loose growth of

* Also at the Autumn Show, Crystal Palace, Sept. 1934.

† The combination *Candida sitophila* is used by Jaczewski in his *Elements de la Mycologie*, p. 196 (1933).

weak hyphae on the surface. The crowded septate hyphae which fill the perithecial cavity at the time the asci start are doubtfully described as paraphyses. They disappear so soon that they would not be observed except in very young perithecia."

Neurospora tetrasperma Shear & Dodge, tom. cit. p. 1027

"Conidial stage (*Monilia tetrasperma* nom.nov.): On corn-meal agar in tubes very scanty whitish growth of mycelia about the upper edge of the agar slant; conidial tufts usually small and scanty, pale salmon; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, nearly hyaline or pale salmon in mass, 8–11 μ in diameter, mostly 8–9 μ.

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed when grown on agar media, smooth or loosely and sparsely soft hairy when superficial, yellowish when young, becoming dark brown to black; 250–300 μ in diameter; wall medium thick, subcoriacious; ostiole obtuse, papillate; paraphyses (?) crowded, septate, collapsing and disappearing as soon as the asci appear; ascospores uniseriate or somewhat overlapping, oblong-elliptical with about 20 longitudinal, sometimes branched ridges, olivaceous at first, becoming dark brown to black, 29–35 × 14–16 μ, mostly 30–31 × 15 μ. This species is homothallic." Collected on charred furze stems in quantity, Bostall Heath, Woolwich, October 1933 (J.R.).

In our cultures conidia were rather sparsely developed. The majority of asci contain four spores c. 35 × 17 μ; some have six or five spores and occasionally three. The small spores vary from 24–31 × 4 μ, the larger ones may be over 40 μ long. We have compared the fungus with a culture obtained from Dr B. Colson (cf. *Ann. Bot.* XLVIII (1934), 211–25) subcultured from one sent to her by Drs Shear and Dodge. The two are identical.

The fungus sent to us in culture by Mr Wilkins agrees with the description given by Shear and Dodge for their *Neurospora sitophila*. There was a certain relief felt when this was realised for it would have been a little unfortunate to have a conidial stage recorded for this country as *Monilia sitophila* and only *Neurospora tetrasperma* as a perfect stage. Mr Wilkins's discovery conveniently disposes of any questioning of Shear and Dodge's statement about *M. sitophila*. "Whether this species is identical with Montagne's fungus it is impossible to determine at present, as the ascospore stage of his plant was not found. This is the common red bread mold of Europe and America and presumably Montagne's species; for practical purposes, therefore, this name may be adopted." The specific epithet of the perfect stage takes precedence over that of the conidial stage but it would be some-

what incongruous to have it open to question whether *Monilia sitophila* was really the conidial stage of *Neurospora sitophila* with a possible upsetting of the nomenclature adopted by Shear and Dodge, and the usual complications rendered a little ludicrous.

Neurospora sitophila Shear & Dodge, tom. cit. p. 1026

"Conidial stage (*Monilia sitophila* Mont.): On corn-meal agar tufted or irregularly pulvinate at first, soon becoming effuse and fluffy; conidial masses varying from pale salmon to orange color; mycelium hyaline, creeping, septate; sporogenous hyphae irregularly ascending, septate, dichotomously branched; conidia catenulate, connected by a narrow isthmus when mature, globose to subglobose, smooth, pale salmon to orange in mass, 10 to 12 μ diameter, mostly 10 to 11 μ.

Perithecial stage: Perithecia gregarious or scattered, superficial or immersed on agar media, smooth or loosely soft, hairy, brown to black subcoriaceous, 200 to 300 μ diameter; ostiole papillate; asci cylindrical, short stipitate, apex with a thickened gelatinous ring, 8-spored, 140–160 × 12–14 μ; paraphyses (?) crowded, multiseptate, filling the perithecium at first, collapsing and disappearing as the asci develop; ascospores overlapping uniseriate, elliptical, with 16 to 17 sometimes branched longitudinal ribs, olivaceous becoming dark greenish black, 20–26 × 10–15 μ, mostly 23–25 × 13–15 μ."

Our cultures produced large quantities of conidia, especially at the top of the culture tube or at the edge of the Petri dish.

The main interest in *Neurospora* is in the occurrence of both homothallism and heterothallism which are connected with the nuclear condition of the ascospores. Shear and Dodge found that *N. tetrasperma* was homothallic and *N. sitophila* was heterothallic. It was found that small spores which occur with some frequency in the asci of *N. tetrasperma* gave strains which did not produce perithecia in the ordinary way but only when mated. Dodge (1927), showed that *N. tetrasperma* had ascospores which were normally binucleate but that the small spores were uninucleate like the spores of *N. sitophila*, thus finding a nuclear explanation of the presence of both homothallism and heterothallism in the one species.

The conidial stage of *N. tetrasperma* gathered on burnt gorse was cultured on Sabouraud's maltose and glucose media and on potato agar. Hyphae and conidia grew very rapidly and numerous well-formed perithecia were formed in about seven days being particularly abundant on the potato agar. Cultures from single ascospores were obtained by placing a few drops of a dilute spore suspension on to agar in a Petri dish. The spores were examined under a microscope and rings made round those which were well isolated. These were then transferred to fresh Petri dishes. The majority of the cultures were homothallic producing perithecia in abundance.

Heterothallic strains were obtained by plating out very small spores. Sterile "sclerotia" developed but no perithecia occurred unless two opposite strains were mated. Strains which were labelled A, X and Y were paired. A plus X and X plus Y always produced perithecia, but A plus Y was invariably sterile (Pl. VI, figs. 1–3). Perithecia usually occurred on one half of the dish only; "sclerotia" appear everywhere.

From the culture sent by Mr Wilkins ascospore cultures were made. These when grown singly produced "sclerotia" but no perithecia. From a series of strains those labelled S, Q and R were mated: S plus Q gave fertile perithecia, S plus R gave fertile perithecia, R plus Q gave "sclerotia" only (Pl. VII).

In both *N. tetrasperma* and *N. sitophila* the sclerotia arise in the same way as the fertile perithecia. Coiled multinucleate hyphae (archicarps) are produced in abundance. These are quickly surrounded by large numbers of intricately woven sheathing hyphae. No antheridia are formed. The "sclerotia" or sterile perithecia usually attain a diameter of about 100 μ but occasionally may be almost as large as the perithecia (Pl. V, figs. 7 and 10).

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EXPLANATION OF PLATES V—VII

Plate V

Neurospora tetrasperma

Figs. 1–5. Developmental stages of archicarps.
Fig. 6. Conidia.
Fig. 7. Sclerotium.

N. sitophila

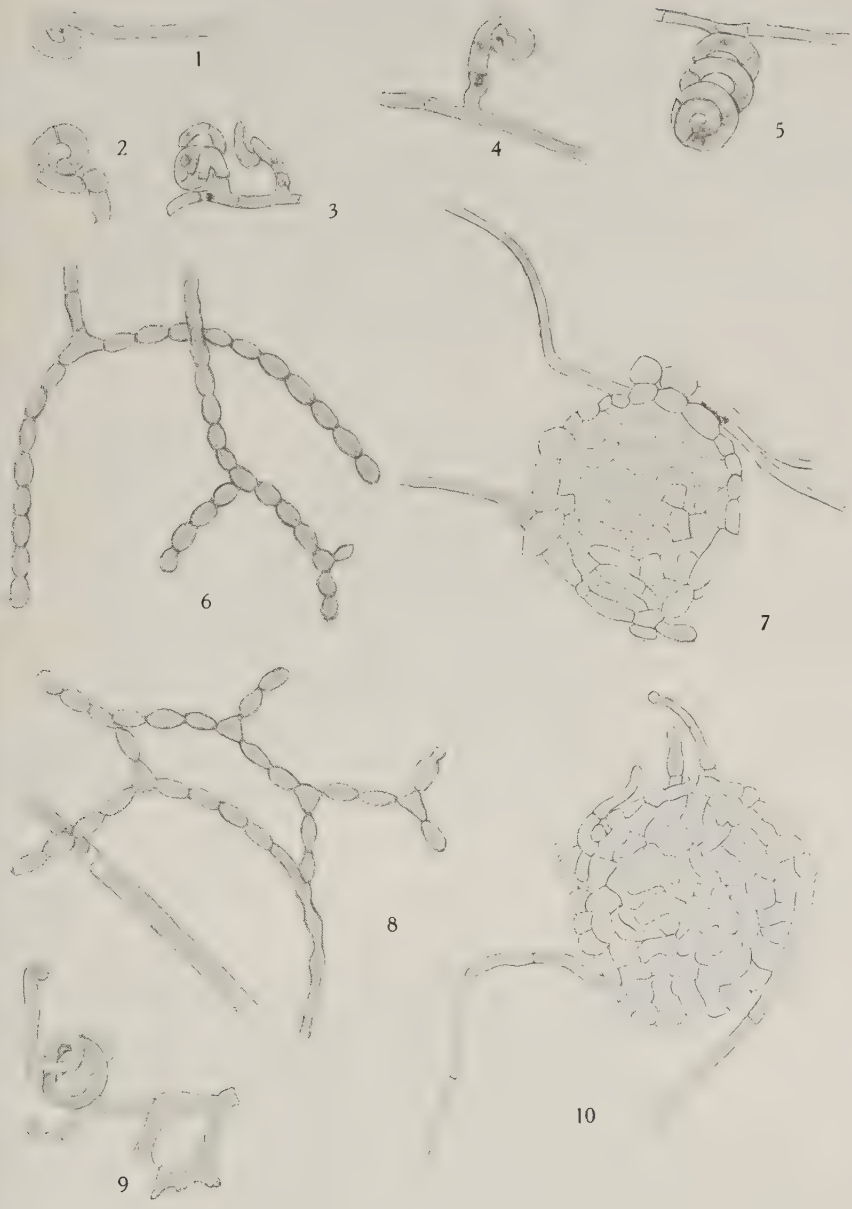
Fig. 8. Conidia.
Fig. 9. Archicarp.
Fig. 10. Sclerotium.

Plate VI

N. tetrasperma. Strains X and A, X and Y, Y and A.

Plate VII

N. sitophila. Strains S and R, R and Q, S and Q.



'Del. C. L. H.'

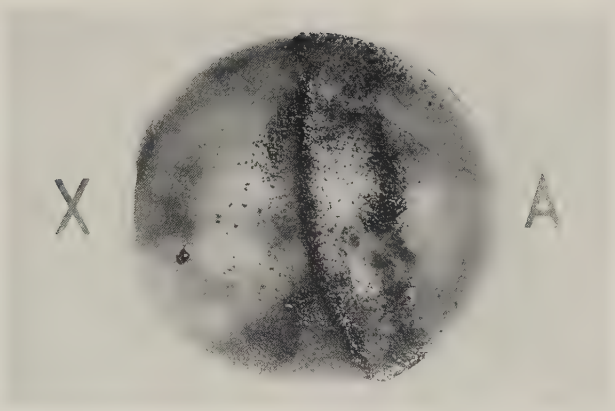


Fig. 1

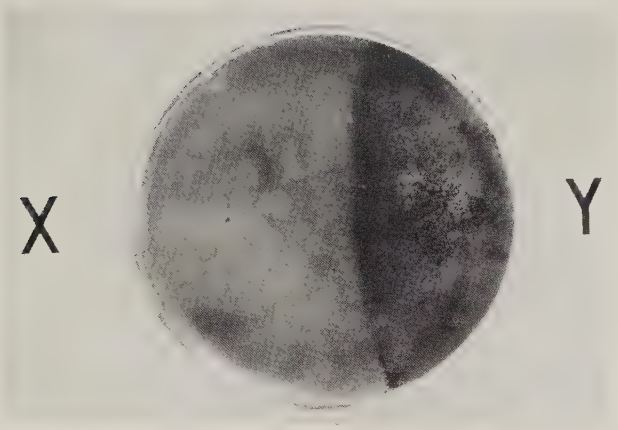


Fig. 2

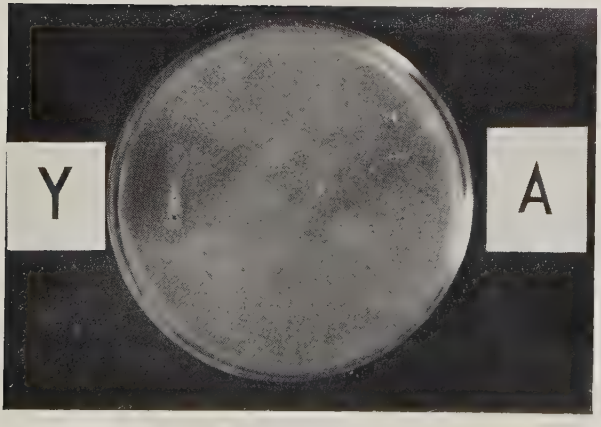


Fig. 3

S + R
R + Q
S + Q

OBSERVATIONS ON SOME BRITISH WATER MOULDS (SAPROLEGNIALES AND BLASTOCLADIALES)

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(With Plates VIII–X and 2 Text-figures)

Introduction

The first attempt to prepare a complete list of British aquatic fungi appears to be due to Massee (20), who recorded eleven species of Saprolegniaceae. Ramsbottom (25) published a new list containing seventeen species of Saprolegniaceae and Leptomitaceae, and Barnes and Melville (1) added nine more species to the records. Sparrow reported the occurrence of *Rhipidium*, *Blastocladia* and *Sapromyces* in a paper read to the British Mycological Society in November 1932, and in a paper read to the British Association at Leicester, in 1933, Barnes recorded *Rhipidium europaeum* and *Sapromyces Reinschii* from another locality. Subsequent to that meeting, Cook and Forbes (7) published a preliminary account of their findings near Bristol, listing the present twenty-two species, of which fourteen were thought to be new records for this country.

Von Minden (21) and Petersen (24) have investigated systematically the aquatic fungi of Germany and Denmark. In America, Coker (3), and his co-workers (4, 5, 6, 12), during twenty years of investigation at the University of North Carolina, have perfected a technique for dealing with these fungi, and have found many new species, as well as many of the species described by earlier workers. These methods have now been applied with considerable success in an investigation of the water moulds of the Bristol district.

Methods

It is well known (13, 18, 23) that aquatic fungi are very susceptible to changes in the conditions of their environment, and that such changes may lead to variation even in those characters which have been regarded as constant and of value in classification. Consequently, in the work now to be described, the same substratum has been used throughout, and, as far as possible, the cultures have been kept under identical conditions.

The methods recommended by Couch (8) have been used in collecting and culturing the fungi.

Rough cultures and preliminary measures

Most natural waters seem to contain aquatic fungi. Such waters, brought into the laboratory in stoppered bottles, placed in crystallising dishes with loosely fitting covers and baited with halves of boiled hemp seeds, usually yield a visible mycelial growth in a few days. After washing with sterile distilled water, and transferring to pots of distilled water—preferably sterile—the mycelium on the hemp seed develops sporangia in a few days and sexual organs after a week or two; identification of the fungus is now possible.

Pure cultures

A piece of hemp seed may bear mycelia of more than one species. These may be identified provisionally, but pure cultures are necessary for the study of a complete life history. Pure cultures are obtained from single hyphae bearing sporangia or oogonia, teased out under the binocular microscope and transferred to plates of malt agar or of corn-meal agar. The mycelium is freed from bacteria by subculturing. Frequently, the fungi do not fruit on agar, and a portion of the mycelium must be transferred to distilled water in order to obtain the reproductive organs.

Special culture methods

Species of such genera as *Rhipidium* and *Blastocladia*, which have a differentiated basal segment attached to the substratum by rhizoids need special treatment. Such species do not grow on hemp seed but they develop well on fruits of rose. The fruits are placed in cylinders of perforated zinc moored by a wire and left in the water for three or four weeks. The fruits are then removed and placed in distilled water. The fungi develop as whitish pustules.

These forms cannot be transferred to other substrata, and pure cultures have not been obtained. Usually, however, a pustule contains only one species. Bacterial contamination is heavy and it is essential to keep these fungi at low temperatures (4–8° C.) if a satisfactory condition is to be maintained. The fruits must be washed frequently during the period of cultivation.

Pythiomorpha, although filamentous, also needs special treatment. It is not found on hempseed, but appears when grapes are used as bait; it has also been found on slices of apple, and twice on dead twigs. When grown on grapes, the fungus is treated in the same way as *Rhipidium*; pure cultures may be obtained on malt agar.

Collections were made during the period September to March, with the exception that *Brevilegnia* was found in August. Although the records refer only to those months, clear evidence of periodicity was shown by the work of Miss G. E. Allen, to whom I am indebted for a supply of the rough material which served as a starting point for the preparation of cultures used for the identification and description of many of the species. Miss Allen made regular collections in the neighbourhood of Bristol, and found that the species occurred in greatest abundance in January.

Systematic

The classification used by Fitzpatrick(11) has been followed, with the exception that Petersen's(24) family, Pythiomorphaceae, has been retained, since a study of the reproductive organs of *Pythiomorpha* shows that Fitzpatrick was not justified in placing this fungus in the genus *Phytophthora*. Almost without exception, it has been found possible to accept the genera and species as they have been defined by Coker(3) and by Kanouse(15, 16, 17).

In the record which follows, the species of each genus are divided into two groups:

(*a*) Species already recorded in this country.

(*b*) Species found for the first time in this country during the present investigation.

Full diagnoses are not given for the species of group A, but observations not agreeing with previous statements are recorded. The species of group B are fully described and the points in which there are differences from older descriptions are noted. Species of either group which were adequately figured by Coker(3) Kanouse(15, 16, 17) or in any other readily accessible account are not figured here, but references are given to the appropriate figures.

Saprolegniaceae

Genus ***Saprolegnia*** Nees v. Esenbeck (1823)

(*a*) *Species already recorded in this country*

1. *Saprolegnia ferax* (Gruithuisen) Kützing, *Phyc. Generalis* (1843) (*Achlya prolifera*, Prings. in *Nova Acta C.L.C.N.C.* XXIII (1851), 395; *Saprolegnia dioica* Prings. in *Jb. wiss. Bot.* II (1860), 206; S. *Thureti* de Bary, *Beitr. Morph. u. Phys. d. Pilze*, IV (1881), 102; *S. bodanica* Maurizio in *Jb. wiss. Bot.* XXIX (1896), 107; *S. esorina* Maurizio in tom. cit. p. 82).

   Figures: Coker, 1923, Pl. 11.

   Found thirty times near Bristol from December to March.

Saprolegnia mixta de Bary in *Bot. Z.* XLI (1883), 38.

   Found three times near Bristol in November.

Saprolegnia ferax usually has antheridia on less than 10 per cent. of the oogonia, whereas *S. mixta* has antheridia on about 50 per cent. of the oogonia. *S. mixta* has been described by many authors since de Bary, and most have commented on the difficulty of regarding *S. ferax* and *S. mixta* as distinct species. The production of antheridia by *S. ferax* is a very variable character, and the same culture may at one time resemble *S. ferax* and at another be indistinguishable from *S. mixta* (Davis (9), Coker (3)).

It appears that *S. mixta* de Bary is really a growth form of *S. ferax* (Gruith.) Kütz., which is an extremely variable species under different environmental conditions. Some material without antheridia was obtained in the collections near Bristol, and other material had oogonia with 50 per cent. antheridia or more. No special factor seemed to be responsible for this variation.

The variability of this species extends to its growth habit and to other features. Von Minden (21) described two forms of *S. Thureti* (*S. ferax* (Gruith.) Kütz.), differing in the following points:

(i) Oogonia terminal, or often on long bent stalks, typically spherical, very rarely cylindrical.

(ii) Oogonia mostly on the ends of very short lateral branches, in regular racemose arrangement; many of the large oogonia, containing numerous eggs, cylindrical in empty sporangia.

The form described by Fischer (10) resembled the second of von Minden's forms, as did also in many respects those of Humphrey (14) and Coker (3). Form (ii) was the more common form found around Bristol, the racemose arrangement of oogonia and the frequent occurrence of cylindrical oogonia being very noticeable. These cultures were usually examined before they were more than a month old. In two experiments, cultures with the appearance of form (ii) were kept in distilled water for three or four months, and by the end of this time the character of the material had changed greatly. Cylindrical oogonia were no longer found, and the oogonia present were borne on side branches that were much longer than those of the former racemose arrangment. As the cylindrical form of the oogonia is due to their origin within old empty sporangia, it is to be expected that old cultures would cease to form such oogonia, since the hyphae have grown beyond the region where sporangia were produced. Young cultures contained oogonia on long and on short stalks, and there was no known reason for this variation. It seems probable that the two forms are not really distinct, and that they merge into one another.

A few young cultures however produced form (i) of von Minden.

(*b*) *Species new to this country*

2. *Saprolegnia monoica* Pringsh. in *Jb. wiss. Bot.* I (1858), 284 (*Diplanes saprolegnioides* Leitgeb, in *Jb. wiss. Bot.* VII (1869), 357, ? *Achlya intermedia* Bail in *Amtl. Ber. 35te Versamml. Deutsch. Naturf. n. Aerzte in Königsberg*, 1860, *Saprolegnia semidioica* Peters. in *Ann. Myc.* VIII (1910), 494).

Figure: Petersen, 1910, fig. 1, p. 520.

Found four times near Bristol in January and February.

Diagnosis. Growth (on hempseed), up to 1 cm. long. *Hyphae* up to 100μ broad at the base. *Sporangia and spores* as usual in the genus; secondary sporangia very numerous and markedly constricted at intervals by mouths of earlier sporangia. *Oogonia* borne racemosely on short, straight or bent stalks, spherical, with conspicuously pitted walls, smooth, 60–110μ diameter, usually 70–90μ; basal wall frequently protruding into the oogonium. *Oospores* 6–22 per oogonium, usually 10 or more, 19–24μ diameter, mostly 21μ, centric. *Antheridia* clavate, androgynous, on all the oogonia, arising from the main hypha or from the oogonial stalk; antheridial stalks branched and often supplying more than one oogonium.

Saprolegnia monoica resembles *S. ferax* very closely in everything except the percentage of antheridia; Coker(3), however, by using suitable nutrient media, showed that the percentage of antheridia in *S. ferax* may be made to vary from 1 to 98. It is difficult to understand how a form of *S. ferax* with antheridia on nearly all the oogonia could be distinguished from *S. monoica* as found near Bristol.

It has been shown that, owing to the variability of the percentage of antheridia, it is not possible to separate *S. ferax* and *S. mixta* as good species. It now seems possible that the very variable *S. ferax* may include among its growth forms one which is indistinguishable from *S. monoica*; if that is so, *S. ferax* and *S. monoica* cannot be regarded as distinct species. An illuminating fact is that while *S. ferax* was most abundant in the collections made from September to November, *S. monoica*, which did not appear until November, became the more frequent in the later months. It seems possible that there are two growth forms whose appearance is controlled by some seasonal factor, such as temperature.

In these fungi a species seems to consist of a number of forms whose features vary over a wide range, merging into one another and often completely overlapping (cf. *Achlya de Baryana*). Such comprehensive species seem to be the smallest units with constant distinguishing characters into which these fungi can be divided. The forms within a species show definite tendencies which they preserve fairly constantly under normal conditions, but they are not true species, since one

form may be induced to assume the characters of another by variations in the nutrition (Klebs (19), Horn (13), Kauffman (18), and other authors). It therefore seems possible that *S. ferax*, *S. mixta* and *S. monoica* may in the future come to be regarded as forms of one species, exhibiting different tendencies, as shown in forma *ferax* (antheridia on 0–10 per cent. of the oogonia), f. *mixta* (antheridia on 50 per cent. of the oogonia) and f. *monoica* (antheridia on 100 per cent. of the oogonia).

2*a*. *Saprolegnia monoica* Pringsh. var. *glomerata* Tiesenhausen in *Arch. Hydrobiol. Plankt.* VII (1912), 261.
Figures: Coker, 1923, Pl. 13.
Found five times near Bristol from January to March.

Diagnosis. *Growth*, *hyphae*, *sporangia* and *spores*, exactly as in the species. *Oogonia* borne racemosely on short side branches which are usually very contorted or bent, 40–80μ diameter, usually 50–60μ, otherwise as in the species. *Oospores* 2–10 per oogonium, usually 3–5, 24–28μ diameter, mostly 26–27μ, centric. *Antheridia* clavate, androgynous, arising from main hypha or from oogonial stalk; antheridial stalks often much branched and contorted. Fertilisation tubes seen.

This variety is distinguished from the species by the smaller oogonia, the fewer and larger eggs, and the peculiarly contorted clumps of side branches and stalks. The present observations have shown, however, that forms intermediate in the size of the oogonia and the number and size of the oospores are frequent. For example:

(*a*) Forms with large oogonia (av. 70–80μ) with a few large eggs (av. 27μ) were obtained three times.

(*b*) Forms with smaller oogonia (50–60μ) with smaller eggs (av. 21μ) were obtained three times.

(*c*) Several forms which did not quite correspond in their dimensions to either the species or the variety were also obtained.

It seems, therefore, that these quantitative characters can no longer be used to distinguish the variety. However, the abundant production of side branches and antheridial stalks, themselves much branched and bent, is a distinctive feature which appears to justify the retention of the variety.

3. *Saprolegnia dioica* de Bary in *Bot. Z.* XLVI (1888), 619 (*S. diclina Humph.* in *Trans. Amer. phil. Soc.* XVII (1892), 109; not *S. dioica* Pringsh. or *S. dioica* Schröter).
Figures: Coker, 1923, Pl. 3.
Found six times near Bristol, from October to January.

Diagnosis. Growth up to 1·5 cm. long. *Hyphae* up to 45μ at the base. *Sporangia* and *spores* as typical in the genus. *Oogonia* terminal or intercalary, sometimes up to 5 or 6 in a chain, large, spherical to pear-shaped, or, when intercalary, barrel-shaped; wall pitted rather infrequently; 45–110μ diameter. *Oospores*, 3–20 per oogonium, usually 8–14, centric, 20–27μ diameter, av. 24μ. *Antheridia* diclinous, clavate, several per oogonium; arising on delicate branches which tend to disappear. *Gemmae* numerous, cylindrical, fusiform or pear-shaped; often in chains.

Genus ***Achlya*** Nees v. Esenbeck, (1823)

(*a*) *Species already recorded in this country*

4. *Achlya colorata* Pringsh. in *S.B. Akad. Wiss. Berlin* (1882), p. 855 (*Achlya racemosa* var. *stelligera* Cornu in *Ann. Sci. nat. Bot.* xv (1872), 22).
   Figure: Coker, 1923, Pl. 32.
   Found four times near Bristol in November and March.

5. *Achlya polyandra* Hildebrand in *Jb. wiss. Bot.* vi (1867), 258 (*Achlya gracilipes* de Bary in *Bot. Z.* xlvi (1888), 635; not *A. polyandra* de Bary, *Beitr. Morph. u. Phys. d. Pilze*, iv (1881), 49).
   Figures: de Bary *Bot. Z.* xlvi (1888), Pl. 10, 2 and 6.
   Found fourteen times near Bristol from October to March.

6. *Achlya apiculata* de Bary in *Bot. Z.* xlvi (1888), 635.
   Figure: Coker, 1923, Pl. 42.
   Found three times near Bristol in October and January.

(*b*) *Species new to this country*

7. *Achlya de Baryana* Humphrey in *Trans. Amer. phil. Soc.* xvii (1893), 117 (*Achlya polyandra* de Bary, *Beitr. Morph. u. Phys. d. Pilze*, iv (1881); not *A. polyandra* Hildebrand).
   Figure: de Bary, *Beitr. Morph. u. Phys. d. Pilze*, iv (1881), Pl. 4.
   Found seven times near Bristol from October to December.

Diagnosis. Growth up to 1·5 cm. long. *Hyphae* up to 100μ at the base. *Sporangia* and *spores* typical for the genus: *sporangia* 350–650 × 30–60μ; *spores* 10–11μ when encysted. *Oogonia* spherical, borne racemosely on short stalks, less than or up to three times the oogonial diameter in length; wall pitted only where the antheridia touch, and not always there; basal wall occasionally protruding into the oogonium; 45–80μ diameter, usually 55–60μ. *Oospores* 2–12 per oogonium, mostly 3–6; 20–24μ diameter, usually 22–23μ, eccentric. *Antheridia* androgynous or diclinous; stalks branched and sometimes

supplying more than one oogonium; one to several per oogonium. Fertilisation tubes seen. *Gemmae* cylindrical, formed by segmentation of the hyphae.

7*a*. *Achlya americana* Humphrey in *Trans. Amer. phil. Soc.* XVII (1893), 116.

Figure: Coker, 1923, Pl. 33.

Found twice near Bristol in December.

Diagnosis. *Growth*, *hyphae*, *sporangia*, *spores* and *gemmae* as in *A. de Baryana*. *Oogonia* as in *A. de Baryana* but with conspicuously pitted walls. *Oospores* as in *A. de Baryana*. *Antheridia* androgynous, arising from the main hyphae or occasionally from the oogonial stalk.

In addition to the forms referred to *Achlya de Baryana* and *A. americana*, intermediate forms have been found on several occasions:

(*a*) A form of *A. americana* which had fewer and more inconspicuous pits than usual, and occasional diclinous antheridia.

(*b*) A form with completely unpitted walls combined with androgynous antheridia.

(*c*) A form with diclinous antheridia and some pitted walls.

The chief distinctions made between *A. de Baryana* and *A. americana* are that the former has unpitted oogonial walls and some diclinous antheridia, whilst the latter has definitely pitted oogonial walls and the antheridia are consistently androgynous.

Horn (13), however, in an investigation of the effects of changes of nutrition and of temperature on *A. polyandra* de Bary (*A. de Baryana* Humph.), found that many characters formerly considered constant were really very variable. His work goes far to eliminate the distinctions between the two species.

Both Petersen (24) and von Minden (21) reduced the status of *Achlya americana* to *A. de Baryana* forma *americana*, and Coker (2) commented on the impossibility of separating the two species. He found, as has been found in the present work, that forms were constantly obtained which combined the characters of the two, so that these forms could not be certainly referred to one or other of the species. It, therefore, appears that *A. de Baryana* and *A. americana* form a parallel pair to *Saprolegnia ferax* and *S. mixta*. Under constant conditions the two forms normally present distinct characters, but they can be made to vary towards one another in culture, and sometimes do so in natural conditions.

Achlya de Baryana is one of the commonest species of the genus in Europe, but up to 1923 (3) it had not been found in America, at least in its typical form. On the other hand, Humphrey (14) stated that *A. americana* was "the most abundant member of the genus and indeed of this family" in the United States, while the only European records of

A. americana, up to 1923, were due to Petersen and to von Minden. This seems to indicate that we are dealing with one species which is common on both sides of the Atlantic, and which tends to develop certain characters in the United States, and rather different characters in Europe.

8. *Achlya Klebsiana* Pieters in *Bot. Gaz.* LX (1915), 486.
Figure: Coker, 1923, Pl. 40.
Found six times near Bristol in October and November.

Diagnosis. Growth up to 1·0 cm. long. *Hyphae* up to 85 μ at the base. *Sporangia* 200–700 × 20–50 μ; secondary sporangia often formed by the outgrowth of part of a hyphal segment cut off below a terminal primary sporangium, sometimes in basipetal succession by repetitions of the process. *Oogonia* in racemes on very short, straight stalks, spherical, sometimes with inward protrusion of the basal wall; pits usually present only where the antheridia touch; diameter 45–60 μ, usually 50–55 μ. *Oospores* 1–13 per oogonium, usually 4–7, eccentric, diameter 19–25 μ. *Antheridia* always diclinous; somewhat elongated and often with foot-like projections touching the oogonia; stalks long, slender and branched. *Gemmae* cylindrical.

This species is very close to *A. de Baryana*. It is distinguished by the very characteristic arrangement of the secondary sporangia, by the slightly smaller oogonia on very short stalks, and by the strictly diclinous antheridia of somewhat different shape.

9. *Achlya caroliniana* Coker in *Bot. Gaz.* L (1910), 381.
Pl. VIII, fig. 1.
Found once near Bristol, in November.

Diagnosis. Growth up to 1·5 cm. long. *Hyphae* up to 70 μ at the base. *Sporangia* about 350 × 30 μ, rarely quite cylindrical. *Spores* 10·5–12 μ in diameter when encysted. *Oogonia* very numerous, small, in a dense raceme on straight stalks which are 1–4 oogonial diameters in length, and sometimes branched at right angles; sometimes spherical, sometimes with one or more papillae; wall unpitted; basal protrusion sometimes present; diameter 30–40 μ. *Oospores* 1–4 per oogonium, usually 1–2, eccentric, diameter 19–22 μ. *Antheridia* may be present on up to 50 per cent. of the oogonia in young cultures, on much branched stalks; androgynous or diclinous; entirely absent from older cultures. *Gemmae* slightly swollen.

Coker(3) first stated that antheridia were absent, but later(4) he reported that in a form obtained from the soil, antheridia were present on 25–40 per cent. of the oogonia; he did not state the manner of origin of the antheridia, which, in the Bristol material, were as often androgynous as diclinous.

10. *Achlya Orion* Coker & Couch in *J. Elisha Mitchell sci. Soc.* XXXVI (1920), 100.
Figure: Coker, 1923, Pl. 35.
Found four times near Bristol in January and February.

Diagnosis. Growth up to 1 cm. long. *Hyphae* up to 60μ at the base. *Sporangia* 100–400 × 20–30μ. *Spores*, diameter 10μ when encysted. *Oogonia* terminal on main hyphae or in racemes on curved stalks; spherical; walls usually unpitted except where the antheridia touch; diameter 30–50μ, mostly 35–40μ. *Oospores* 1–2 per oogonium; eccentric; 24–40μ diameter, mostly 27–33μ. *Antheridia* androgynous, clavate, arising from main hyphae or from oogonial stalks; present on most oogonia.

11. *Achlya radiosa* Maurizio in *Mitt. d. dtsch. Fischerei-Vereins*, VII, 1 (1899), 57 (*A. decorata* Petersen in *Ann. Myc.* VIII (1910), 522; *A. asterophora* Minden in *Krypt. Fl. d. Mark Brandenburg*, V (1915), 549).
Pl. VIII, fig. 2.
Found four times near Bristol in January and February.

Diagnosis. Growth up to 1 cm. long. *Hyphae* up to 90μ at the base. *Sporangia* 250–400 × 25–35μ; secondary sporangia sparse. *Spores*, diameter 10–11μ when encysted. *Oogonia* numerous, terminal on main hyphae or in racemes on short stalks; closely set with conical pointed hollow outgrowths; walls unpitted; diameter, without spines 36–56μ, with spines 45–75μ; spines 8–18μ long and 7–13μ wide at the base. *Oospores* 1–2 per oogonium, eccentric, 30–43μ diameter, mostly 35–38μ. *Antheridia* always present, androgynous, on short curved branches from the oogonial stalks; tuberous, applied near the base of the oogonium by their distal ends; 1–2 per oogonium; fertilisation tubes very frequently seen.

12. *Achlya oblongata* de Bary in *Bot. Z.* XLVI (1888), 646.
Figure: Coker, 1923, Pl. 47.
Found five times near Bristol from September to February.

Diagnosis. Growth usually exceeding 1·5 cm. *Hyphae* up to 130μ at the base. *Sporangia* 350–600 × 30–70μ. *Spores*, diameter 10–11·5μ when encysted. *Oogonia* terminal or on rather long lateral branches, rarely intercalary; oval or pear-shaped; walls unpitted; basal wall occasionally protruding into the oogonium; 75–150 × 45–100μ. *Oospores* 2–15 per oogonium, mostly 5–10, subcentric, diameter 22–31μ, average 27μ. *Antheridia* diclinous, one or more per oogonium, on branching stalks; tuberous. *Gemmae* cylindrical or pear-shaped.

12*a*. *Achlya oblongata* de Bary var. *gigantica* nov.var.
Pl. VIII, fig. 3.
Found twice near Bristol in January.

Diagnosis. *Growth* 2 cm. or more. *Hyphae* up to 150μ at the base. *Sporangia* as in the species. *Spores*, diameter 12·5μ when encysted. *Oogonia* in racemes on long lateral branches; oblong, pear-shaped or cylindrical; often with a pointed apex, walls unpitted; very large, 90–250 × 50–100μ. *Oospores* 1–8 per oogonium; wall about 2μ thick; 36–49μ diameter; frequently not maturing; very dark when young, and structure difficult to observe, subcentric. *Antheridia* diclinous, on most oogonia.

This remarkable variety resembles the species in nearly all respects except in the outstanding size of the oogonia and oospores; the rather cylindrical pointed shape of many oogonia is also characteristic. As compared with the species, a fully grown culture contains fewer mature oospores, of which the structure is rather obscure.

Achlya oblongata var. *gigantica* nov.var.

A typo differt oogoniis eximie magnis 90–250 × 50–100μ, saepe cylindricis acuminatis; oosporis majoribus 36–49μ diam. paucioribus (singulis ad octonis).

13. *Achlya megasperma* Humphrey in *Trans. Amer. phil. Soc.* XVII (1893), 118.
Figure: Coker, 1923, Pl. 44.
Found six times near Bristol from January to March.

Diagnosis. *Growth* up to 1·5 cm. long. *Hyphae* up to 150μ at the base. *Sporangia* 400–600 × 40–70μ. *Spores*, diameter 11·5–13μ when encysted. *Oogonia* in racemes on short, straight stalks; spherical to rather oval; walls unpitted, 3·5μ thick; protrusion of basal wall frequent. *Oospores* 2–9 per oogonium, usually 3–6; 43–60μ diameter, mostly 45–50μ, subcentric. *Antheridia* androgynous or diclinous, on about 50 per cent. of the oogonia; stalks branched and often supplying more than one oogonium. Fertilisation tubes seen. *Gemmae* cylindrical.

The oogonial wall is rather thinner at the point of contact with the antheridium. Numerous much branched side shoots arise from the main hyphae; they resemble antheridial stalks, but usually remain sterile.

14. *Achlya recurva* Cornu in *Ann. Sci. nat. Bot.* XV (1872), 22.
Pl. VIII, fig. 4.
Found eight times near Bristol from October to March.

Diagnosis. *Growth* up to 2 cm. long. *Hyphae* up to 80μ wide at the base. *Sporangia* about 355 × 30μ. *Spores*, diameter 14–16μ when

encysted. *Oogonia* numerous, in loose racemes on short or long stalks which are usually bent or curved right over; spherical, with many short conical outgrowths; walls unpitted; diameter 45–95 μ, including spines, usually 70–80 μ; spines 7–17 μ long, 8–11 μ wide at the base. *Oospores* 1–15 per oogonium, usually 5–8; 25–35 μ diameter, mostly 27–29 μ; subcentric. *Antheridia* androgynous or diclinous; stalks slender and often branched.

15. *Achlya apiculata* de Bary var. *prolifica* Coker & Couch, *The Saprolegniaceae*, 1923, p. 127.
Figure: Coker, 1923, Pl. 43, 2–7.
Found once near Bristol in November.

Diagnosis. Growth not exceeding 1 cm. *Hyphae* up to 100 μ wide at the base. *Sporangia*, *spores* and *gemmae* as in the species. *Oogonia* exceedingly numerous; as in the species but smaller and with more frequent apiculi; 40–75 μ, usually 50–60 μ. *Oospores* fewer than in the species, 1–2 per oogonium. *Antheridia* as in the species.

Genus ***Dictyuchus*** Leitgeb in *Bot. Z.* XXVI (1868), 502.

(*a*) *Species already recorded in this country*

16. *Dictyuchus monosporus* Leitgeb in *Jb. wiss. Bot.* VII (1869), 357.
Figures: Couch, *Ann. Bot.* XL (1926), Pl. 37 and 38.
Found once near Bristol in September.

On several occasions a growth has been identified as a species of *Dictyuchus* by means of the sporangia, but the oogonia necessary for specific determination could not be obtained even after prolonged cultivation. Since Couch (8) has shown that heterothallism occurs in *Dictyuchus*, it is possible that these non-sexual forms, with similar forms found by other workers, may represent the antheridial or oogonial strains of a heterothallic fungus.

Genus ***Calyptralegnia*** Coker & Couch in *J. Elisha Mitchell sci. Soc.* XLII (1927), 219.

(*b*) *Species new to this country*

17. *Calyptralegnia achlyoides* Coker & Couch *loc. cit.* (*Thraustotheca achlyoides* Coker & Couch in *J. Elisha Mitchell sci. Soc.* XXXIX (1923), 112).
Pl. IX, fig. 6.
Found ten times near Bristol from September to March.

Diagnosis. Growth up to 2·0 cm. long. *Hyphae* up to 150 μ wide at the base. *Sporangia* primarily terminal, secondarily cymose, cylindrical, not much wider than the hyphae; 400–900 × 40–70 μ; tips blunt;

apex completely removed as a lid at dehiscence. *Spores*, diameter when encysted 12–14μ. *Oogonia* terminal or in racemes, on straight or curved stalks which may be less than, or up to four times the oogonial diameter; spherical; walls unpitted; diameter 60–120μ. *Oospores* usually 1–2 per oogonium, rarely 3–4, subcentric, 55–70μ diameter. *Antheridia* androgynous, arising frequently from the oogonial stalks; present on most oogonia.

Spore discharge may not occur immediately after dehiscence but the spores may protrude from the opening, giving a characteristic appearance. When the spores are discharged, they escape rather slowly, with an irregular trickling movement. Nearly always, up to half the spores are left inside the sporangium. The discharged spores form a loose mass at the mouth of the sporangium, each encysted as an irregular polyhedron. In a minute or less, reniform zoospores with two lateral cilia emerge from the cysts; the cysts may also germinate by means of a germ tube. Spores left in the sporangium also encyst, and they do not usually develop further, though the cysts may sometimes yield zoospores which leave the sporangium.

Coker and Couch stated that oogonia were rare in their cultures and that attempts to induce their formation by the use of various media were unsuccessful. The material found near Bristol yielded oogonia abundantly.

Genus ***Brevilegnia*** Coker in *J. Elisha Mitchell sci. Soc.* XLII (1927), 207.

(*b*) *Species new to this country*

18. *Brevilegnia diclina* Harvey in *J. Elisha Mitchell sci. Soc.* (XLII), 1927. Pl. IX, fig. 5.
Found once in Bristol in August; in soil from a fern-pot in a hot greenhouse.

Diagnosis. *Growth* up to 1 cm. long. *Hyphae* up to 40μ wide at the base, usually up to 25μ. *Sporangia* primarily terminal, secondarily cymose, clavate, long or short, 100–250 × 10–30μ, sometimes with only a single row of spores. *Spores* spherical to subspherical, average diameter 12μ. *Oogonia* on long, twisted, very narrow and branched stalks (average diameter 5μ), which look like a secondary mycelium of small hyphae among the larger hyphae; small, irregular in shape, being rounded with many protuberances; 18–30μ diameter, mostly 20–25μ; walls unpitted. *Oospores* solitary, eccentric, 15–22μ diameter, average 18μ. *Antheridia*, diclinous, clavate, present on only a few oogonia.

LEPTOMITACEAE

Genus ***Leptomitus*** Agardh, 1824

(*a*) *Species already recorded in this country*

19. *Leptomitus lacteus* (Roth.) Agardh, *Syst. Alg.* 1824 (*Conferva lactea* Roth. (*Beitr. z. Bot.* 1789); *Saprolegnia lactea* Pringsh. in *Jb. wiss. Bot.* II (1860), 205; *Apodya lactea* Cornu in *Ann. Sci. nat. Bot.* XV (1872), 5).
Figure: Coker, 1923, Pl. 58.
Found eight times near Bristol from October to February.

Genus ***Rhipidium*** Cornu in *Bull. Soc. Bot. France*, XVIII (1871), 58.

(*b*) *Species new to this country*

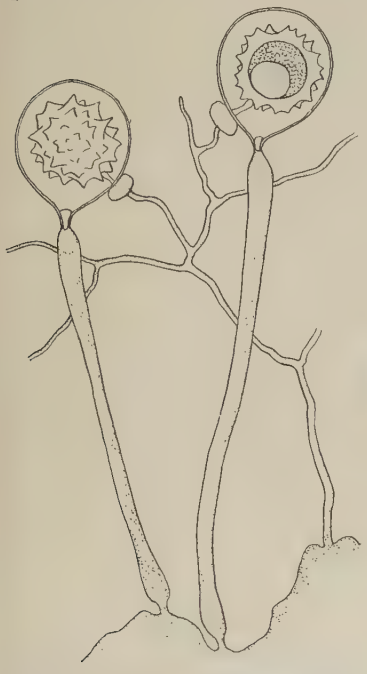
20. *Rhipidium europaeum* Minden in *Krypt. Fl. Mark Brandenburg*, V (1912) (*Rhipidium continuum* Cornu and *R. interruptum* Cornu in *Ann. Sci. nat. Bot.* XV (1872), 15).
Text-fig. 1; Pl. X, fig. 8.
Found seven times near Bristol from September to January.

Diagnosis. Thallus of a large, irregularly cylindrical basal portion, somewhat lobed at the apex and sometimes dichotomously branched. The broad rounded lobes bear very narrow branches, constricted at the point of origin and sometimes along their length; the branches have a bulbous swelling just above the basal constriction. The thallus is attached to the substratum by numerous branched rhizoids, and contains coarse granular protoplasm. Basal portion 400–900 × 30–90 μ; wall up to 15 μ thick; branches 100–500 × 9–14 μ. *Sporangia* terminal on the branches, with a constriction immediately beneath them; secondary sporangia arise on lateral branches developing from the primaries immediately beneath the sub-sporangial constriction; sympodial groups result from repeated branchings; sporangia oval, with apical dehiscence, 45–70 × 25–47 μ. *Spores* seen only as rather large rounded masses inside the sporangia. *Oogonia* terminal on narrow branches with sub-oogonial constrictions, spherical to somewhat pear-shaped; walls smooth and unpitted; 47–57 μ diameter. *Oospores* solitary, walls thick (up to 17 μ), sculptured externally, with ridges and points, eccentric, 40–50 μ diameter. *Antheridia* small 12–16 μ wide, tuberous, applied to the base of the oogonium; diclinous, on much branched stalks.

20*a*. *Rhipidium europaeum* Minden var. *compactum* nov.var.
Pl. X, fig. 11.
Found three times near Bristol in November and January.

Diagnosis. Thallus of a very short, broadly cylindrical basal cell

bearing a large number of broad, subdivided lobes, which spread out and around the short stalk in a compact bunch; these lobes bear the narrow branches which are rather shorter than in the normal form. Rhizoidal system relatively well developed, rhizoids stout with rounded tips. *Sporangia* as in the species, 50–60 × 25–35 μ. *Oogonia* pear-shaped, smaller than in the species, 40–45 μ diameter. *Oospores* smaller than in the species, 28–36 μ diameter. *Antheridia* as in the species.



Text-fig. 1. *Rhipidium europaeum* Minden. Oogonia with antheridia (× 175).

Text-fig. 2. *R. americanum* Thaxter. Narrow branches bearing sporangia and oogonia (× 110).

Rhipidium europaeum Minden var. *compactum* nov.var.

A typo differt cellula basali brevissima multilobata; filamentis aliquantum brevioribus; rhizoideis bene evolutis crassis ad apices rotundatis; sporangiis 50–60 × 25–35 μ; oogoniis pyriformibus minoribus 40–45 μ diam.

21. *Rhipidium americanum* Thaxter in *Bot. Gaz.* XXI (1896), 320.
Text-fig. 2.
Found three times near Bristol in November and January.

Diagnosis. Thallus of a large, much branched basal cell, showing successive dichotomy, and attached by numerous rhizoids. The upper

lobes bear many narrow branches constricted at their point of attachment, and constricted beneath the reproductive organs borne upon them. Basal cell, from origin of rhizoids to origin of first lobe, 500–1000 × 60–110 μ; lobes up to 800 μ long. Narrow branches 200–800 × 10–15 μ; rhizoidal system up to 2 mm. long. Walls up to 5 μ thick. *Sporangia* terminal, with secondary cymes, ovoid, 40–110 × 25–40 μ. *Spores* not seen. *Oogonia* terminal, spherical, smooth-walled, 40–50 μ diameter. *Oospores* solitary, with thick, ridged wall, eccentric, 35–45 μ. *Antheridia* androgynous, 11–14 μ diameter, arising from the antheridial stalks, immediately below the oogonial constriction, rounded, applied to the base of the oogonium. Fertilisation tubes seen.

PYTHIOMORPHACEAE

Genus ***Pythiomorpha*** Petersen in *Ann. Myc.* VIII (1910), 494.

(*a*) *Species already recorded in this country*

22. *Pythiomorpha gonapodioides* Petersen, *loc. cit.*
Pl. IX, fig. 7.
Found about twelve times near Bristol throughout the year.

Petersen (24) first described this species, but he did not obtain the sexual stage. Von Minden (21) observed oogonia in mixed cultures, and Kanouse (15) obtained the sexual organs in cultures from single spores. My cultures have oogonia, resembling in many respects those described and figured by Kanouse, and, in addition, they yielded oogonia and antheridia of more definite structure; these are regarded as the successfully matured sexual organs.

The oogonia are borne on short lateral branches of the intramatrical mycelium. They are spherical, with a single, thin, unpitted wall. Mature oogonia are dark brown, those with indefinite contents are yellowish brown, and empty oogonia are pale yellow; diameter 26–35 μ. The oospores are solitary, completely filling the oogonium, with very thick, smooth walls; they are dark brown, centric, and average 30 μ diameter. Antheridia are diclinous and clavate; they appear to be present on all oogonia, one to each as a rule; they are 11–15 μ wide. The apex of the antheridium is attached close to the base of the oogonium. Mature antheridia are dark brown, empty ones are colourless.

The oogonia resembling those described by Kanouse contained evenly distributed protoplasm, completely filling the oogonium, and of indefinite structure. Some had a single thin wall, others contained inside, the thicker wall of the oospore. These oogonia are regarded as immature, or as showing premature arrest of development.

Antheridia occurred on almost all the oogonia in my cultures, whereas Kanouse found them on only about 10 per cent. of the

oogonia. This is not surprising if her material was immature. Kanouse did not comment on the dark brown colour of the sexual organs; this feature is not strongly marked in immature stages.

Mature oogonia show only two walls, the thin oogonial wall and the much thicker wall of the oospore; Kanouse recorded three walls in her material. Her figures, however, give the impression that only two walls were present, since in her drawings the oogonia are surrounded by *three* lines only. It has not been found possible to confirm the account given by Kanouse of the structure of the wall of the sporangia. She states that the wall becomes differentiated into an outer and an inner layer, separated by a hyaline layer; this has not been seen in the Bristol material, in either mature or empty sporangia.

BLASTOCLADIALES

Blastocladiaceae

Genus ***Blastocladia*** Reinsch. 1878

(*a*) *Species already recorded in this country*

23. *Blastocladia Pringsheimii* Reinsch. in *Jb. wiss. Bot.* xi (1878), 298. Pl. X, fig. 10.

Found six times near Bristol from September to January.

This species has been recorded from a single doubtful specimen obtained by Barnes and Melville(1); therefore, although the present finds do not constitute a new British record, it seems suitable to give a full diagnosis.

Diagnosis. Thallus consisting of a stout basal cell, either simple or much lobed apically; the lobes may be branched, their apices becoming swollen and bearing the reproductive organs. Numerous branching rhizoids attach the base of the thallus to the substratum. The wall is thick and scaly, and the lobes are reticulately marked by scars of old sporangia. Protoplasm rather dark, containing many spherical oily globules. Simple or branched sterile hairs, with bulbous bases, arise from the basal cell or from its lobes. The whole plant, including the rhizoids, may be up to 2 mm. long; the basal cell is 400–1000 × 50–200μ; the lobes are 240–400 × 80–160μ; the wall is up to 8μ thick; the sterile hairs are 2–5μ diameter. *Sporangia* sessile on the swollen apices of the lobes, cylindrical, fusiform to nearly ovoid, thin-walled, with a basal septum; dehiscence apical, with exit papilla when ripe; the empty sporangia fall away; 150–250 × 30–47μ. *Spores* seen only in sporangia, or rounded off owing to interruption of spore discharge; diameter 11–14μ; apparently they do not emerge in a vesicle. *Oogonia* sessile, oval or rounded, with a thin wall; average diameter 50μ. *Oospore* solitary, completely filling the oogonium, with

a very thick, regularly pitted wall; contents very oily; the oospore escapes from the oogonium before germination.

Summary

An examination has been made of the natural waters of the Bristol district, using methods recommended by American workers. Twenty-two species of aquatic fungi have been found, fourteen of which have not before been recorded in this country; these have been fully described.

Species found

(*a*) Species already recorded in this country: *Saprolegnia ferax* Kützing, *Achlya colorata* Pringsheim, *A. polyandra* Hildebrand, *A. apiculata* de Bary, *Dictyuchus monosporus* Leitgeb, *Leptomitus lacteus* Agardh, *Pythiomorpha gonapodioides* Petersen and *Blastocladia Pringsheimii* Reinsch.

(*b*) Species new to this country: *Achlya de Baryana* Humphrey, *A. Klebsiana* Pieters, *A. caroliniana* Coker, *A. Orion* Coker & Couch, *A. radiosa* Maurizio, *A. oblongata* de Bary, *A. oblongata* var. *gigantica* nov.var., *A. megasperma* Humphrey, *A. recurva* Cornu, *A. apiculata* var. *prolifica* Coker & Couch, *Calyptralegnia achlyoides* Coker & Couch, *Brevilegnia diclina* Coker, *Rhipidium europaeum* von Minden, *R. europaeum* var. *compactum* nov.var., *R. americanum* Thaxter.

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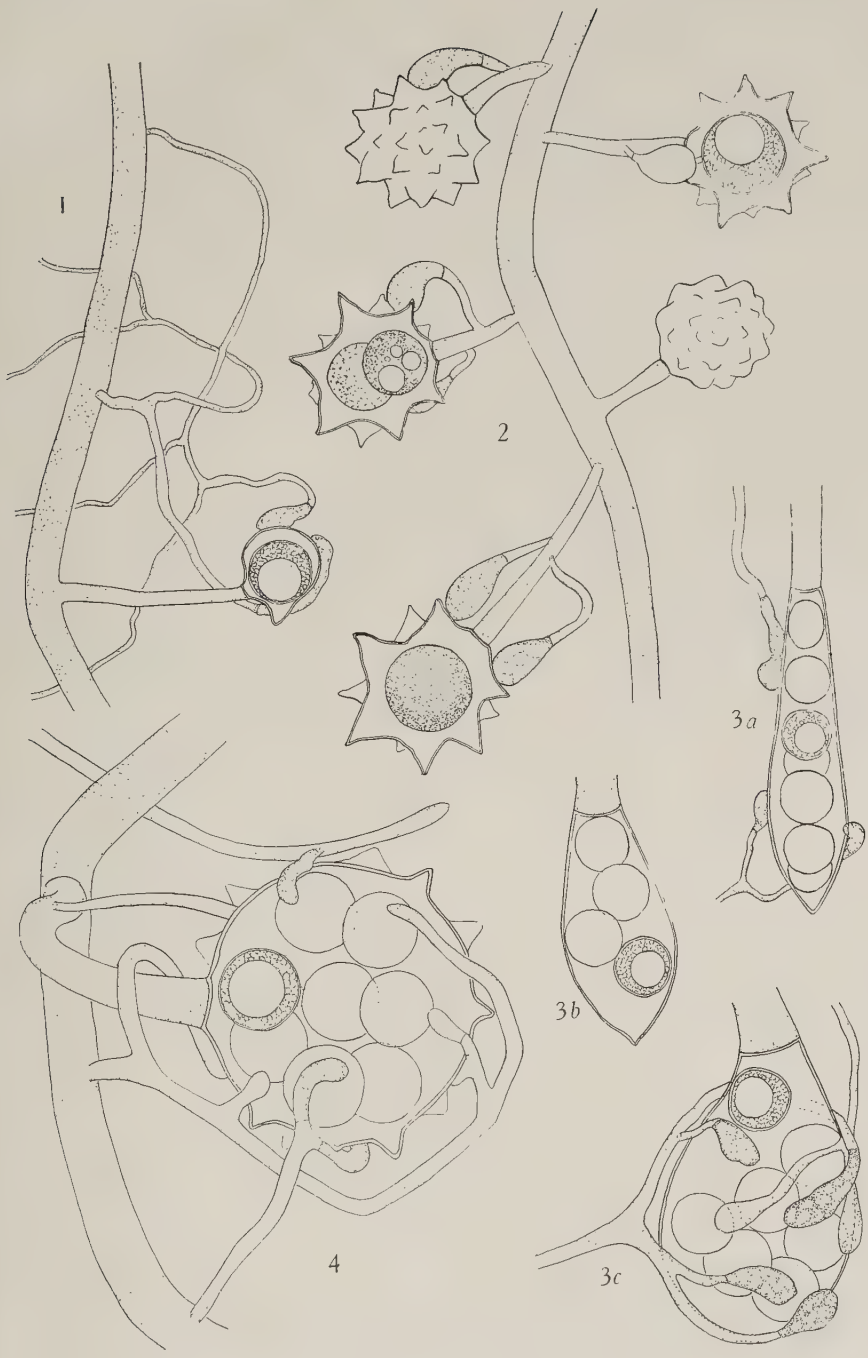
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EXPLANATION OF PLATES VIII—X

Plate VIII

Fig. 1. *Achlya caroliniana* Coker. Oogonium with androgynous antheridia (× 175).
Fig. 2. *A. radiosa* Maurizio. Hypha with oogonia and antheridia of various ages (× 125).
Fig. 3. *A. oblongata* var. *gigantica* nov.var. (*a*), (*b*) and (*c*). Oogonia and antheridia (× 82).
Fig. 4. *A. recurva* Cornu. Oogonium with antheridia (× 175).

Plate IX

Fig. 5. *Brevilegnia diclina* Harvey. (*a*) Oogonia of various ages (× 285). (*b*) Ripe sporangium. (*c*) Spores freed by disappearance of sporangium wall. (*d*) As in (*c*), with spores in linear arrangement. (*e*) Germinating spores. (*b*), (*c*), (*d*) and (*e*) × 175.
Fig. 6. *Calyptralegnia achlyoides* Coker & Couch. (*a*) Oogonia with antheridia (× 110). (*b*) Sporangia, showing types of spore-behaviour (× 50). (*c*) Encysted spores, with one emerging from its cyst. (*d*) Swimming spores. (*e*) Spores germinating by hyphae. (*c*), (*d*) and (*e*) × 375.
Fig. 7. *Pythiomorpha gonapodioides* Petersen. Oogonium with antheridium (× 750).

Plate X

Fig. 8. *Rhipidium europaeum* von Minden. Mature thallus showing sporangial stage (× 70).
Fig. 9. *R. americanum* Thaxter. Young thallus with developing sporangia (× 50).
Fig. 10. *Blastocladia Pringsheimii* Reinsch. Mature thallus with sporangia and oogonia (× 50).
Fig. 11. *R. europaeum* var. *compactum* var.nov. Young thallus with sporangia (× 70).

N.B. Except in (11), the rhizoidal systems are for the most part missing.

AN EXPERIMENTAL AND CYTOLOGICAL STUDY OF THE LIFE HISTORY OF ENDOPHYLLUM SEMPERVIVI

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(With 23 Text-figures)

The genus *Endophyllum* was one which aroused interest at an early period in the investigation of the plant rusts, by reason of its special short-cycled nature, for only the two spore forms, spermogonia and aecidia, occur. A further interest was added when it was found that, although the spores which developed in association with the spermogonia were borne in sori that had morphologically the appearance of aecidia and were therefore termed aecidiospores, they behaved like teleutospores in giving rise to a promycelium and sporidia.

The first study of *Endophyllum Sempervivi* was undertaken by de Bary (6). He stressed the apparent anomaly of an aecidium producing spores, the subsequent germination of which showed them to be the equivalent of teleutospores. He figured the germination of one of these, the infection of the host tissue by the basidiospores, and he reported the presence of a perennial mycelium. He also described certain plants of *Sempervivum* infected with *Endophyllum* on which he had never seen the development of spermogonia, although the aecidia were produced with normally developed and fertile spores. It is unfortunate, in view of more recent work on this fungus, that the nuclear content of these spores was not known. The continued seasonal occurrence of such a form is easily explained by the perennating mycelium.

Later Maire (10) described the cytological phenomena accompanying the germination of the aecidio-teleutospores, as they had come to be named. He reported that these were binucleate and that the two nuclei passed into the germ tube without fusion, and there divided to give a four-segmented promycelium.

It was not until 1914 that Hoffmann (8) published his more comprehensive study of the complete life history of the fungus. He reported the initiation of the diplophase by equal cell fusion at the base of the aecidial primordium as in other rusts, although the point at which the fusion occurred was not so well marked as in certain of the

forms previously investigated, nor was the evidence produced so convincing. The binucleate cells thus formed cut off binucleate cells which divided to give rise to intercalary cells and aecidiospores. Thus far, the behaviour of the spore-producing primordium was that of a typical aecidium, but it was established that, instead of these binucleate spores continuing the diplophase, fusion of the conjugate nuclei took place as soon as the spore was detached from the chain. A reduction division might follow this fusion immediately or during the germination of the spore, and as this was followed at once by a second division, the mature spore might appear quadrinucleate. The four nuclei so formed were later separated by wall formation in the promycelium and each passed out to a basidiospore.

Moreau (17) substantiated this work, but also discovered a different course of events for a strain of *Endophyllum Sempervivi* (13) which he found on *Sempervivum* in a cemetery near Bagneaux. Here the two nuclei in the young aecidiospores did not fuse but underwent conjugate division, and the spore thus became quadrinucleate. Subsequently two of these nuclei degenerated, and later the two remaining might fuse. No mention was made of spermogonia in this form.

There is thus considerable conflict of evidence centred round the nuclear events in the aecidiospore, and this is only intensified by comparison with events in what appear to be nearly related forms.

Endophyllum Euphorbiae-sylvaticae on *Euphorbia amygdaloides* was investigated by Sappin-Trouffy (20) and described in his comprehensive review of the Uredinales. He found no fusion in the aecidiospore, nor did he consider that there was evidence for a reduction division, as both the nuclei passed into the promycelium and divided conjugately, although the four nuclei so formed became separated in the promycelium by their cross walls. "Par conséquent il n'y a pas de raison pour identifier cette germination avec celle d'une téleutospore comme on l'a fait jusqu'à ce jour." The value of these observations is enhanced by the fact that in all other rust genera he had studied, Sappin-Trouffy found a nuclear fusion in the teleutospore which to him indicated fertilisation; in *Endophyllum* his results were therefore contrary to expectation.

Later, Moreau (14, 17) described a strain of *Endophyllum Euphorbiae-sylvaticae* which was uninucleate throughout its development. The single nucleus in the aecidiospore divided to give a four-nucleate mycelium. No spermogonia were reported.

Poirault (18, 19) found a uninucleate form of *Endophyllum Centranthi-rubi*. There were no cell fusions in the aecidial primordium. Here, however, the nucleus underwent only a single division and the promycelium was consequently only two-celled and abstricted two sporidia.

Maire (10) found a further variation in *Endophyllum tuberosae*. The

aecidiospores were at first binucleate, but became uninucleate by degeneration of one of the nuclei. The persistent nucleus passed into the promycelium, and was cut off from the body of the spore by a septum. A nuclear division followed and resulted in a two-celled promycelium, but since the nucleus of the lower compartment degenerated, a single sporidium only was abstricted from the tip of the promycelium.

Thus in what is at present regarded as a single genus, there is considerable variation in the development, and it may be necessary to conclude with Moreau: "Pour nous, le nom d'*Endophyllum* n'est pas un nom générique, il ne désigne pas un groupe naturel constituant une unité systématique, il désigne un type de développement caractérisé par la germination des écidiospores en promycelium."

The present study of *Endophyllum Sempervivi* was started primarily to investigate the function of the spermatia, but since such cytological variations had been reported for this species it was considered desirable to work out the developmental history of the particular strain of the fungus under investigation.

Material and methods

Material of *Endophyllum Sempervivi* was first obtained in February 1932 through the kindness of Mr J. Ramsbottom. It was growing on plants of *Sempervivum tectorum* and *S. Webberi*. At this time a considerable infection was apparent; the spermogonia appeared as innumerable minute red specks, whilst the aecidia showed as white spherical pustules of considerable size below the epidermis of the leaves. During the second and third seasons observations were again made on these plants, on a further supply sent by Mr Ramsbottom and also on those plants which had been inoculated with sporidia in the summers of 1932 and 1933. Generally speaking, the infection of these latter plants was lighter than in the others. *Sempervivum tectorum* was used exclusively as the host plants in the infection experiments. In the first season healthy plants of this species were obtained from the Royal Holloway College Botanical Garden where the work was being done, and in the second season Dr B. Barnes very kindly provided a most liberal quantity.

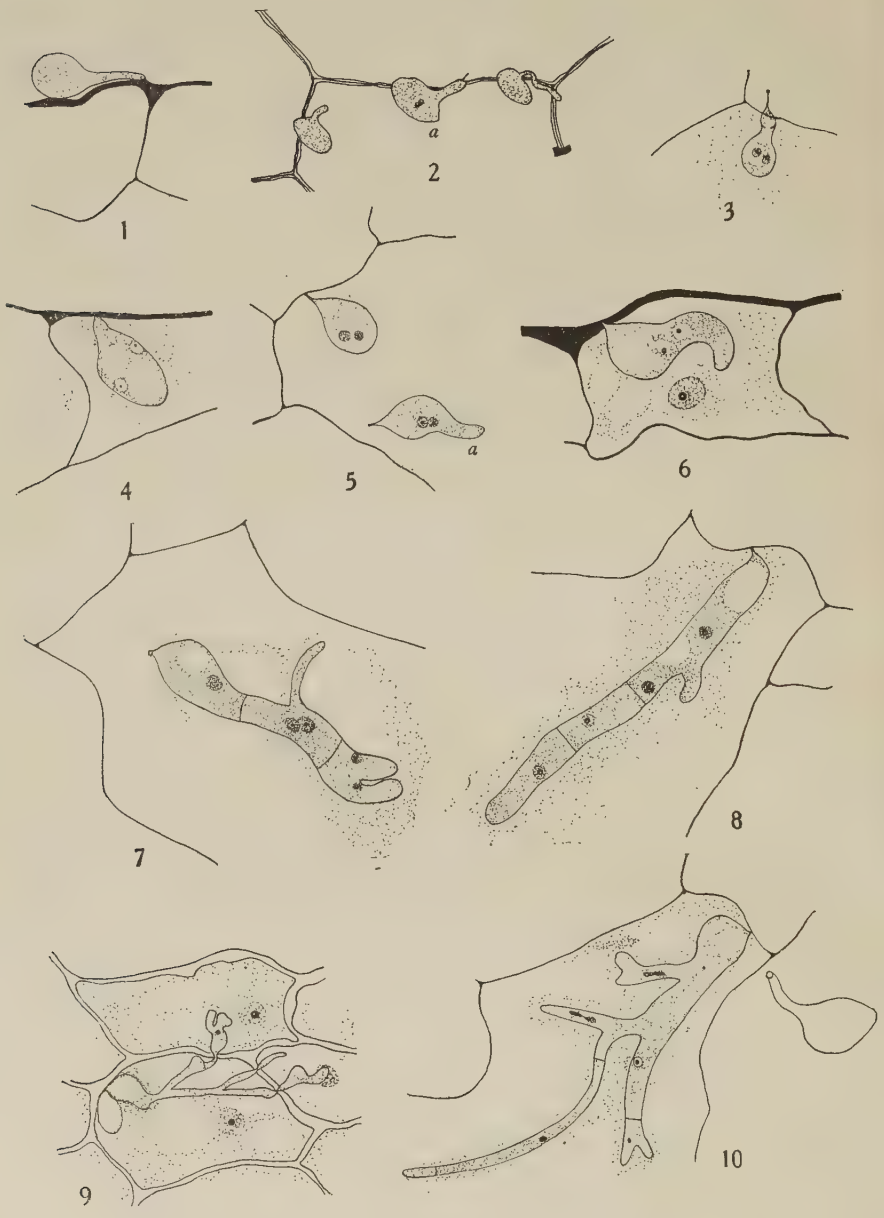
As in the other rusts studied, mono-sporidial and multi-sporidial infections were made. As the mycelium is perennial it was early realised that only a single sporidium per plant could be used in inoculation, to test the homothallic nature of the fungus. The methods for making these inoculations were exactly described in an earlier paper for *Puccinia Malvacearum* (4). A slight difference was made in the preparation of the inoculum. The aecidiospores were germinated in moist chambers on agar films and the sporidia were picked up directly from these films.

For cytological investigations, material was fixed in Allen's Bouin, Flemming's weaker solution and 2B.D.(4) as used at the John Innes Horticultural Institute. Sections were cut at thicknesses from 4μ–8μ and stained in iron alum haemotoxylin or Gram's iodine gentian violet, with Congo red, erythrosin, or light green in clove oil as a counter stain. All methods gave similar results.

The life cycle

The aecidiospores of *Endophyllum Sempervivi* are formed at the beginning of the growing season of the host plant, and germinate as soon as the aecidia are open, to give a promycelium which abstricts sporidia. Out of doors this usually takes place towards the beginning of April and the spores remain viable until the early days of June. In the summer of 1932 spores were germinated successfully from April 15th until June 12th. On plants grown in a slightly heated greenhouse, however, in 1933 and 1934, the aecidia matured much earlier and germination of the spores was obtained as early as March 1st, but no material was germinable after the first week of May.

The sporidia are at first uninucleate, but they become binucleate by a precocious nuclear division even whilst attached to the sterigma. If they fall on the surface of the host plant under the most favourable conditions of temperature and moisture, they will send out a germ tube (Fig. 1). This is usually formed by elongation of the hilum of the spore, and during the process vacuolation of the spore protoplasm occurs although the nuclei remain in the body of the sporidium (Fig. 2*a*). From the tip of this germ tube a penetration-hypha is put out which pierces the cuticle and wall of the epidermis of the host. This minute penetration-hypha often traverses the wall for a considerable distance and through it the contents of the spore pass (Fig. 3). During transit the nuclei must undergo distortion. The precocious nuclear division to give two nuclei of less bulk is possibly correlated with the necessity of passing through so small an aperture. It may be, too, that if one nucleus becomes damaged the second one can carry on development. Once through to the cell lumen, the tip of the penetration-hypha expands forming a minute spherical swelling in which the two nuclei can be seen (Fig. 3). As this vesicle increases in size vacuolation occurs and a little later the part remote from the point of entry becomes differentiated as a thinner type of hypha (Figs. 5*a*, 6). The primary infection-hypha reaches this stage of development within twenty-four hours. During the second day it becomes septate. The end nearer the point of entry retains its expanded form and becomes more and more vacuolate as the hypha grows in size (Fig. 7). Although the binucleate nature of the sporidium has often been reported in other rusts the problem as to how the binucleate hypha becomes uninucleate has not been satisfactorily solved. Deposits of tannin in the



Text-figures 1–10

epidermal cells of the *Sempervivum* leaf render observation difficult, but in this species of *Endophyllum* it seems that either one nucleus only divides and that between these two nuclei a septum is formed so that the cell nearer the point of entry is binucleate and the other uninucleate (Fig. 8) or *vice versa* (Fig. 7). As a matter of fact there is considerable variation in the distribution of nuclei and in the septation of this primary infection-hypha. When it is three or four cells in length each of the cells puts out a lateral branch of rather less diameter than that of the parent cell (Fig. 7). These lateral branches grow rapidly and soon penetrate the wall and invade the surrounding tissues (Fig. 9). Those which enter neighbouring epidermal cells frequently end in a haustorium (Fig. 9), but the mycelium thus formed is composed of uninucleate cells.

Instead of forming spermogonia and aecidia immediately, the intracellular mycelium becomes partly intercellular and passes through the leaf towards the base, and there enters the cortex of the underground stem. This occurs fairly rapidly. For example, a plant inoculated on April 15th showed hyphae in all parts of the leaves infected, including the base by May 5th. The leaves were then removed, to determine whether the fungus had yet entered the stem. In the spring of 1934 this plant showed no infection, so that it was evident that the fungus had not passed into the stem.

Subsequently the mycelium perennates in the underground stem but does not approach the extreme tip. In consequence of this, the new leaves formed at the centre of the rosette between May and the end of the growing season are free from disease. At the end of the year the mycelium passes upwards to the growing point of the host. If a longitudinal section is cut through this region towards the end of November or the beginning of December, a considerable quantity of mycelium can be distinguished, which is characterised by its irregular form and numerous and stout haustoria (Fig. 11). It does not approach the meristematic region of the tip nor does it invade the vascular tissue.

LEGENDS TO TEXT-FIGURES 1–10

Fig. 1. Transverse section of epidermal cell of *Sempervivum tectorum* with a germinating sporidium. × 2000.
Fig. 2. Sporidia on the surface of the host cells. The centre one shows a short germ tube and the fine penetration-hypha. × 1400.
Fig. 3. The spherical vesicle in the host cell. Note the point of entry and the fine penetration-hypha. × 2000.
Figs. 4, 5, 6. Similar binucleate stages; in Fig. 6 the elongation of the infection-hypha has started. × 2000.
Fig. 7. A three-celled stage. The centre cell is putting out a lateral branch. × 2000.
Fig. 8. Another three-celled stage. × 2000.
Fig. 9. Young infection invading adjacent cells. Note the haustoria. × 1400.
Fig. 10. Similar infection. The empty sporidium can be seen on the right. × 2000.

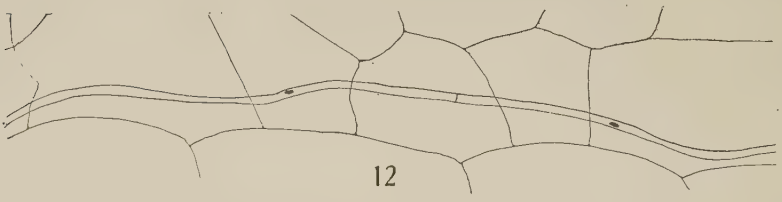


Fig. 11. Perennating mycelium in the cortex of the host. Underground stem. × 1400.
Fig. 12. Hypha in leaf at the beginning of the year. Note the unbranched mycelium. × 1400.
Fig. 13. Mycelium in the subepidermal region of the leaf. The mycelium is grouped beneath stomata. × 760.

The renewal of activity of the mycelium in the spring coincides with the growing season of the host. An early spring or higher artificial temperature will cause the *Sempervivum* plants to sprout early. The first outward signs of the disease are the slightly yellower colour and abnormal elongation of the leaves. The mycelium in the leaves is thinner than that of the underground stem and possesses fewer haustoria. It is characterised by a marked growth in the direction of the longer axis of the leaf (Fig. 12), and the cells, though greatly elongated, are uninucleate. No hyphal fusions were observed in the material examined.

The accompanying figures from the leaves of a healthy and a diseased plant respectively are of interest as showing the effect of the fungus on the leaf at this stage. Figs. 14*a* and 14*b* show that there is a marked difference between the form of the epidermal cells. In the healthy leaf (Fig. 14*a*) the walls appear corrugated, whilst in the diseased leaf (Fig. 14*b*) these corrugations have almost disappeared owing to the elongation of the cells in a sagittal direction. In transverse sections (Fig. 15*a*, *b*) the cells of the healthy plant appear slightly larger than those of the diseased one, but in longitudinal sections (Fig. 16*a*, 16*b*) there is a most striking difference in size shown, which accounts for the considerable elongation of the diseased leaf. Hypertrophy is caused by changes in size and shape of the individual cells rather than in an increase in number.

Subsequently the hyphae become localised in the subepidermal layer of the leaf. There is a considerable massing of hyphae in the substomatal spaces (Fig. 13). There seem to be three possibilities for the further development of these knots of hyphae; they may function as primordia of spermogonia, they may function as primordia of aecidia, or they may remain as stomatal hyphae.

The hyphae which protrude through stomata are in every respect similar to the receptive hyphae described by Andrus (3) for *Uromyces appendiculatus* and Allen (1, 2) for *Puccinia triticina* and *P. coronata*. They were of very frequent occurrence in the earliest stages of the disease. A connection could sometimes be traced between these hyphae and other branches of the mycelium connected to haustoria. This suggests that the hyphae are not trichogynes, since a trichogyne would be attached to a reproductive structure and not to a vegetative organ such as a haustorium.

When a hyphal knot is destined to form a spermogonium the mycelium branches repeatedly at close intervals and the branches become arranged approximately parallel to one another, though owing to their tapering form they converge to a common centre (Fig. 17). The occurrence of spermogonia below stomata is most marked. There appears to be no sheathing peridium, but the circle of hyphae at the extreme edge become differentiated as paraphyses. At

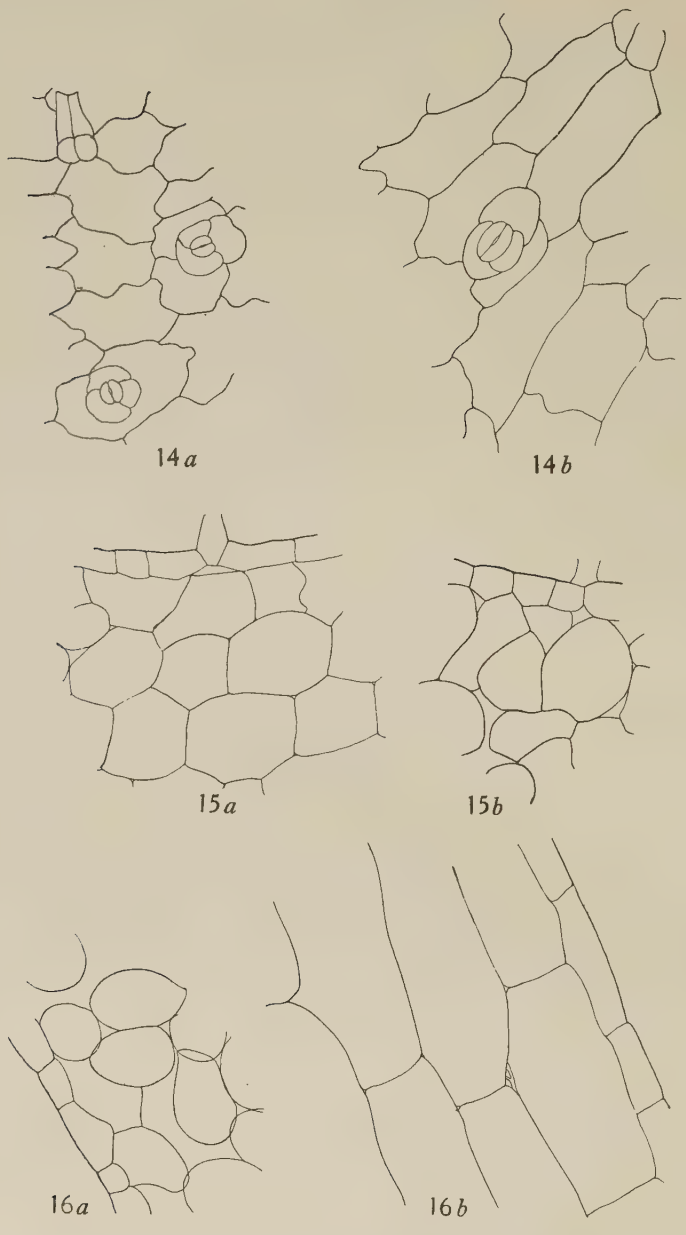
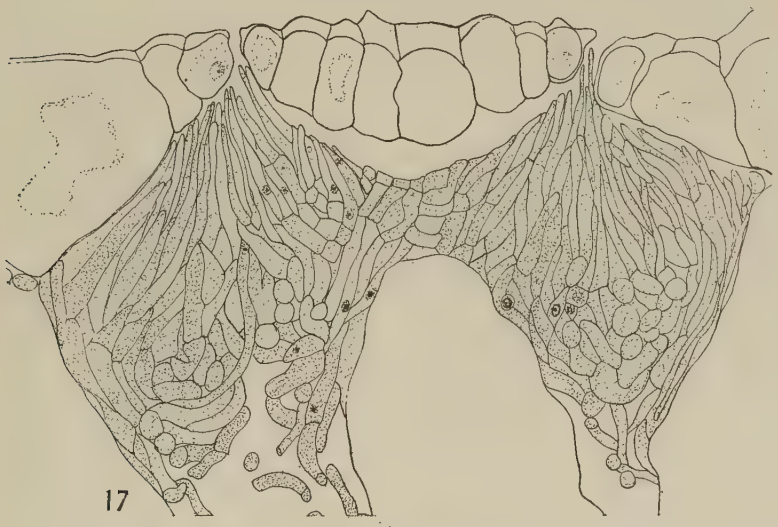


Fig. 14*a*, *b*. Epidermal strips of a healthy and a diseased leaf of *Sempervivum Webberi*.
Fig. 15*a*, *b*. Transverse sections of healthy and diseased leaves.
Fig. 16*a*, *b*. Longitudinal sections of a healthy and a diseased leaf respectively.



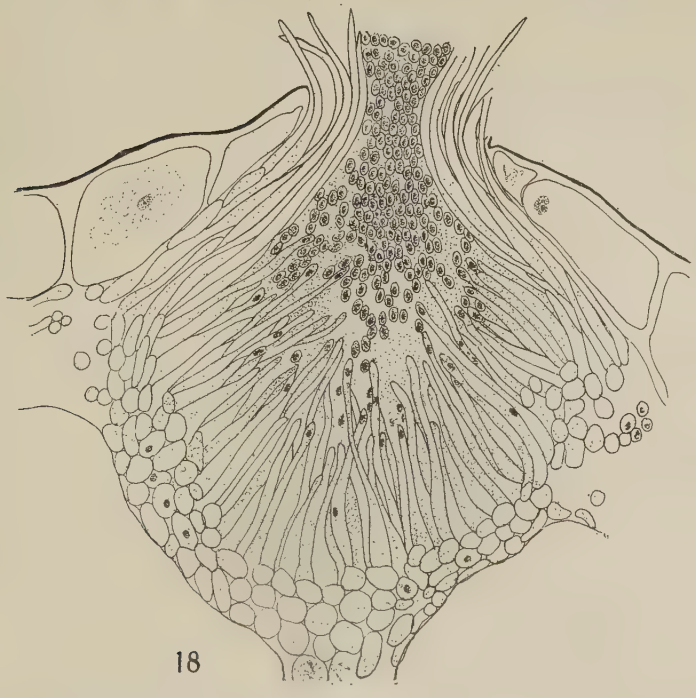


Fig. 17. Two young spermogonia. Note their relation to the stomata. × 760.
Fig. 18. Mature spermogonium with sheathing paraphyses and many spermatia. × 760.

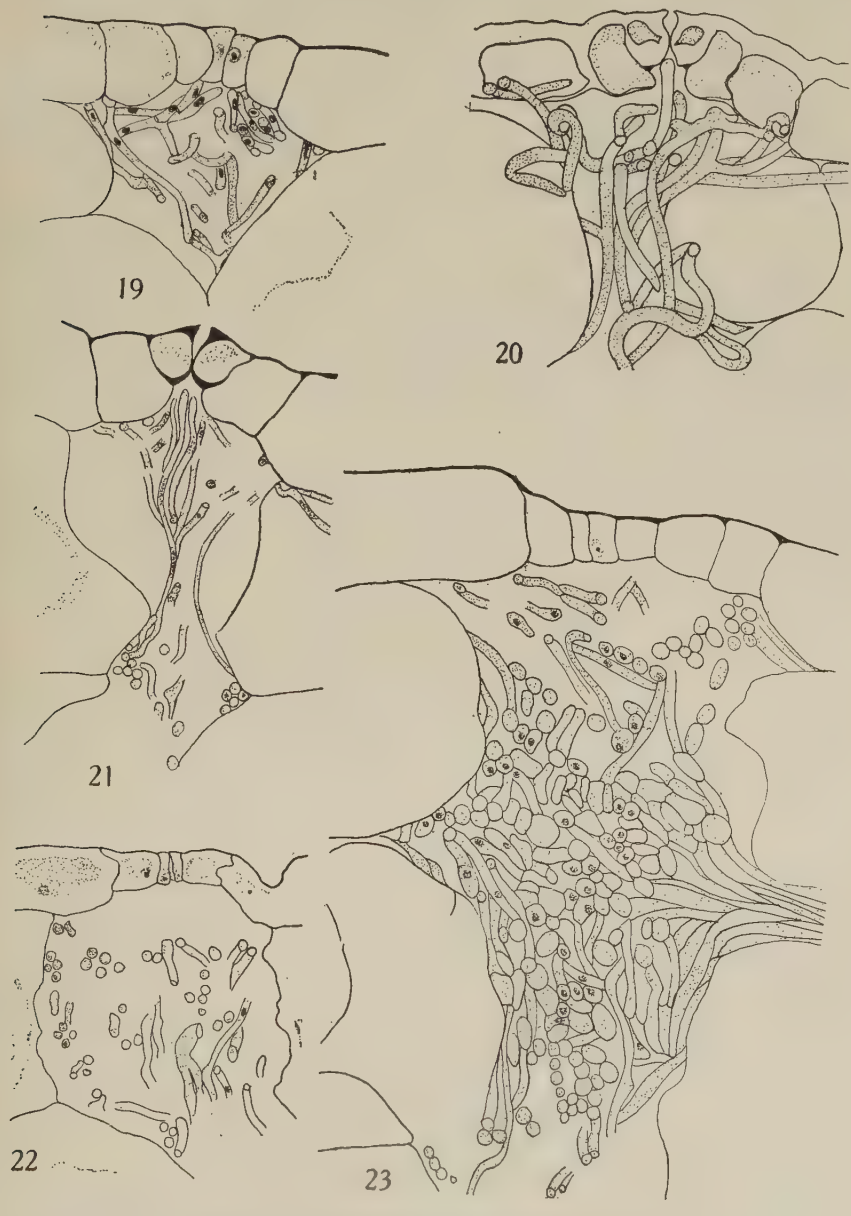
first the spermogonia appear as minute yellow dots beneath the epidermis, but later they assume a bright red shade owing to the colour of the paraphyses, which protrude through the ostiole, and when aged become dark brown and then black. The spermatia are abstricted from the spermatiophores in the usual way by budding, and are rather blunt with the large nucleus which is so characteristic of the spermatium (Fig. 18). There seemed to the naked eye to be little spermogonial nectar but sections of fixed material through ripe spermogonia show masses of spermatia adhering together in spherical drops. A mature spermogonium is about 200 μ in diameter.

In 1933 the first spermogonia were found as minute specks on February 7th and by February 15th these were abstricting spermatia.

Aecidial primordia usually accompanied these, forming small whitish flecks beneath the epidermis. These were composed of less tightly packed wefts of uninucleate mycelium. The hyphae were stouter than those of the spermogonia or the perennating mycelium (Fig. 23). Towards the end of February the primordia had increased greatly in size, and a differentiation could be seen between the three or four layers of cells adjacent to the leaf tissues and the inner weft of mycelium. Cytological examination showed all these hyphae to be uninucleate, and those of the inner region more vacuolate.

Later in the month, certain cells at the base of the aecidium appeared richer in protoplasmic content than the rest and more active. It was difficult to determine whether these hyphae were of new growth or whether they were due to the differentiating tissues, but in view of their subsequent history, the first alternative seems more probable. The component cells were uninucleate.

At this stage the white colour of the primordia was replaced by a rusty tinge. It is interesting to recall that a similar change of colour marked the change from the uninucleate to the binucleate condition in *Puccinia Malvacearum* and has been noted also in *P. Poarum*. Binucleate cells were found mingled with the uninucleate ones, in the tightly packed mass of tissue at the base of the aecidium. Hoffmann (8) figured nuclear migration and remarked that the points at which it took place were less definitely marked than in other rusts. In the present investigation a few possible nuclear migrations have been seen. Once the cells become binucleate, they divide rapidly, the nuclei undergoing conjugate division. Each of these cells is an aecidiospore initial and divides further to give an aecidiospore and an intercalary cell. Quadrinucleate cells may be seen occasionally in the chains. These appear to be cells in which wall formation had not been completed after conjugate division. Meanwhile the more loosely woven tissue which had previously filled the aecidium breaks away to make room for the advancing column of cells enclosed in the peridium. Subsequently the aecidiospores become rounded off by



Figs. 19, 20. Young spermogonia. Fig. 20 was drawn from living material. × 760.
Fig. 21. Hyphae making their way to a stoma. × 760.
Fig. 22. Young aecidium. Note the wider more loosely woven hyphae. × 760.
Fig. 23. Slightly older aecidial weft, still uninucleate. × 760.

thick walls. Amongst these are seen quadrinucleate cells, which may be regarded as spores in which precocious meiosis had taken place. Their thickened walls seemed to differentiate them from those described above. Uninucleate aecidiospores in which fusion of the conjugate nuclei had occurred were not of frequent occurrence, and from this it would seem that there is a very short resting stage before the fusion nucleus undergoes meiosis.

There are two further points of interest to note: firstly, the large number of non-viable spores which frequently appeared white externally, and secondly, the association of the aecidia with stomata. Sometimes as many as three stomata were seen in one section.

The pressure of the ripe aecidiospores causes the peridium to split. There is no definite ostiole in this rust. Frequently the aecidia are visited by small insects, which can sometimes be seen feeding on the spores.

Carefully fixed material of germinating aecidiospores reveals that meiosis occurs in the promycelium, and all stages of these divisions have been obtained. It is suggested that the four-nucleate cells noted above, in which it was thought meiosis might have occurred, are non-viable, because no germ tubes have been seen which enclosed more than a single nucleus in the young stages.

Experimental work

Craigie's(7) work on *Puccinia Helianthi*, *P. graminis* and other rusts suggests that the spermatia of these fungi are functional, although how they function has not yet been satisfactorily demonstrated. The evidence is well known and is, briefly, that pustules of mono-sporidial origin form only spermogonia, unless spermatia of a complementary nature are added to them. The importance of the study of the development of the rust fungi in single-spore culture has thus been revealed. The presence of a perennating mycelium in this rust (*Endophyllum Sempervivi*) renders it necessary that these mono-sporidial inoculations should be confined to a single sporidium per plant, and theoretically in such cultures one would expect to get only spermogonia the following spring. If more than one sporidium were introduced, there is a possibility of the inter-mixing of the mycelia in the root stock. A certain number of such inoculations were attempted, but were considerably limited in number by greenhouse accommodation, and it was felt that another method of tackling the problem might give better results.

The aim of the experimental work was to determine whether aecidia of a normal type developed if the intervention of the spermatia were eliminated. The plants of *Sempervivum tectorum* were favourable objects for such treatment, as the volume of the leaf occupied by the fungus was small compared with the leaf itself, and so

the spermogonia could be treated without causing too much injury to the leaf tissue. Consequently, as soon as the first signs of elongation were seen in the infected plants at the beginning of the year, these were watched carefully for the appearance of spermogonia. As soon as these were seen as tiny specks beneath the epidermis of the leaf they were removed.

In the first group of experiments this was effected by burning them out with a red-hot needle. Care was taken to heat the needle well between each prick, so that there was no possibility of any "mixing" effect, and the spermogonia were treated before they broke through the epidermis. As controls in this experiment, an undiseased plant of *Sempervivum* was treated in the same way to see the effect of the hot stabs on the tissue of the leaf, and a diseased plant was left untreated. The plants were examined each day and developing spermogonia were removed.

Three plants were treated in this way:—in plant A all the spermogonia were removed, in plant B those on the upper surface only and in plant C those on the under surface. The following tables summarise these results. In Table I the daily development of spermogonia is recorded for plant A in which all the spermogonia were removed. In Table II the weekly counts of spermogonia and aecidia are given for plants A, B, C and the Control.

These tables show the following points clearly: (1) in all plants spermogonia developed quite rapidly in spite of treatment, and (2) the greater development of spermogonia and aecidia always took place at the lower surface. The first signs of aecidial primordia were seen on February 10th, and they gradually enlarged. By February 19th the colour of the aecidia had changed to yellow and by February 21st the first ones were open.

This experiment is open to two main criticisms. In the first place it may be argued that the normal physiological processes of the leaf have been interfered with and consequently changes introduced which are not conducive to the further development of the fungus. In those plants in which the spermogonia were removed from the one surface only, no difference in the time of appearance or ripening of the aecidia was detected. The second criticism is that since these plants were in a greenhouse with other infections, spermatia might have been carried by insects and other agencies to the plants from which the spermogonia had been removed and so assured the development of aecidia, not through the spermogonia since they had been removed, but by means of the "receptive" hyphae. Another plant was therefore treated in the same way: all the spermogonia were removed, but the plant was isolated from any possible infection in the laboratory, and aecidia were formed.

The second method was that of treating the leaf surfaces with a

Table I. *The daily development of spermogonia and aecidia in Plant A*

Date	Number of spermogonia	Number of aecidia
February		
7	37	—
8	59	—
9	90	—
10	135	—
11	165	—
12	105	—
13	81	32
14	51	—
15	5	—
16	16	—
17	13	—
18	—	—
19	27	—
20	30	42
21	57	—
22	49	—
23	9	—
24	35	—
25	—	—
26	—	—
27	1	69
28	—	—
March		
1	—	—
2	—	—
3	—	—
4	—	—
5	—	—
6	—	79

Table II. *Weekly counts of spermogonia and aecidia on upper and lower surfaces of five leaves*

Date ...	Feb. 13th		Feb. 20th		Feb. 27th		Mar. 6th		
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Total
Plant A	All spermogonia removed by the hot needle method								
Spermogonia	88	178	91	197	122	230	124	232	356
Aecidia	16	5	18	10	21	16	25	23	46
Plant B	Spermogonia removed on the upper surface only								
Spermogonia	145	238	128	265	130	278	130	278	408
Aecidia	45	121	50	111	52	119	53	126	179
Plant C	Spermogonia removed on the lower surface only								
Spermogonia	—	—	86	195	88	215	88	215	303
Aecidia	—	—	23	40	27	43	27	43	70
Control	(For four leaves)								
Spermogonia	95	146	165	174	197	198	197	198	395
Aecidia	11	6+*p**	34	43	44	38	38	45	83

* *p*=primordia of aecidia which appear as whitish specks.

smear so that the spermatia set free from the spermogonia were held in place and could not mix. Two kinds of smear were used, (*a*) one which would keep the spermatia in place and (*b*) one which would kill them as well as prevent them from being dispersed. Vaseline and liquid paraffin were used for the first type. As in the above experiment, three plants were used. In one both leaf surfaces were smeared, in a second the upper surface only and in a third the lower surface only In the plants treated with vaseline, only one application was made, for it was realised that repeated application might cause mixing of the spermatia and yet not be definitely toxic to them.

In the plants in which both surfaces were smeared no complete development of the fungus was obtained. This seems due to the disturbance of the normal physiological processes of the leaf rather than to the elimination of the work of the spermatia, for the plants became unhealthy. In those in which one surface only was treated, spermogonia and aecidia were formed profusely in the plants treated with vaseline. The liquid paraffin seemed to have a more injurious effect than the vaseline, as it ran down the leaves and collected at the crown of the plant.

The two toxic smears used were (*a*) a fine suspension of shellac in alcohol used for fixing pencil and charcoal drawings and supplied by Rowney's under the trade name "Fixatif" and (*b*) a smear containing a 0·01 per cent. mercuric chloride solution in such quantities of liquid and solid paraffin as to give a fluid of suitable consistency. Its adherence to the leaf surface was ensured by the addition of glycerine. In the experiment with "Fixatif" this was sprayed on the plant daily; the aecidial primordia were seen on February 20th and they developed normally. In the plants treated with mercuric chloride daily the one in which both sides of the leaf were treated died. The other two plants in which the upper and lower surfaces were treated, respectively, formed aecidia on both surfaces. It is interesting to note that Stevens(21) found a $N/6400$ or 0·002 per cent. solution of mercuric chloride sufficiently toxic to prevent the germination of *Uromyces* spores, so that it seems safe to assume in this experiment that development of the aecidia was completed without the intervention of spermogonia.

It remained to determine if the aecidia formed in all these experiments were of the normal type, and if the spores were viable and germinated normally. Comparative examination of carefully fixed material of the control plants and those experimentally treated revealed no difference in development, all the spores arising from binucleate basal cells. The spores subsequently became uninucleate and always germinated to give a four-celled promycelium, abstricting four sporidia.

From these experiments one definite conclusion can be reached.

Aecidia can complete their development without the intervention of spermatia. The initiation of the binucleate stage may be effected by hyphal fusions. Whether this is the usual method still remains to be proved, by evidence showing that ripe aecidia are not developed in mono-sporidial culture. In a rust like *Endophyllum Sempervivi*, with a perennating mycelium, the possibilities of such fusions appear to be greater than in one in which the infection is only local. During the present investigation, however, no binucleate cells have been seen prior to the development of the aecidia.

Summary

1. The sporidia of *Endophyllum Sempervivi* are precociously binucleate.

2. Entrance to the host cell is gained by penetration of the epidermal wall by a fine hypha.

3. The infection vesicle is at first a spherical binucleate sac.

4. Elongation of the part most remote from the point of entry occurs, and subsequent septation takes place.

5. Further growth results in the production of a mycelium of uninucleate cells which is inter- and intra-cellular and passes down to the base of the leaf where it enters the stem.

6. No hypertrophy of the leaves occurs at this stage.

7. Perennating mycelium spreads through all the tissues of the stem except the vascular tissue and the apical meristem, and there passes the winter.

8. On renewal of activity in the spring, the mycelium passes to the leaves and causes hypertrophy by elongation.

9. It becomes massed in the subepidermal tissues, especially in the substomatal spaces.

10. Emergent hyphae are frequently seen protruding through the stomata.

11. Spermogonia are the first organs to be developed and occur markedly below stomata.

12. These are accompanied by wefts of aecidial primordia composed of uninucleate cells.

13. Later a secondary growth appears at the base and the hyphae become binucleate.

14. The diplophase may be initiated by nuclear migration.

15. The nuclei of each binucleate cell divide conjugately and from the aecidiospore mother cell formed an aecidiospore and intercalary cell are produced.

16. The spores may be quadrinucleate whilst still enclosed in the peridium.

17. They germinate to give rise to a three-septate promycelium and abstrict four sporidia.

18. Experiments made to determine the function of the spermatia show that the mature aecidia can be formed when the spermatia are (*a*) removed by burning out with a hot needle, or (*b*) kept in position by the application of a smear, *e.g.* vaseline, liquid paraffin, "Fixatif" or a solution of mercuric chloride.

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SOME FURTHER NOTES ON THE PRESERVATION OF PETRI DISH CULTURES

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Four years ago I described a method of preserving Petri dish cultures of fungi (*Trans. Brit. mycol. Soc.* xv (1930), 93–5), but since then experience has revealed some difficulties that had not before been encountered. The manner in which these may be overcome, together with some slight improvements in the technique, are now recorded as a supplement to the previous paper.

Firstly, very old cultures have been found not to adhere well to the wax. The reason for this is not clear. The agar, which is thickest at the edge as it runs up the side of the glass, is more or less rounded at the bottom rim as moulded by the Petri dish, and besides this, in old cultures the surface of the agar is usually covered with a thick growth of mycelium which hinders drying; these factors may prevent the agar when permeated with hyphae from taking a good grip on the wax. Or possibly the real cause may lie in a physical change in the agar itself, perhaps simply the partial drying up which occurs in old cultures. But whatever the reason may be, there is a tendency for the agar to curl away from the wax in drying, and to prevent this, two methods have been adopted. The first is to cut off the edge of the cultures at an angle of 45°, the top of the cut being innermost; this should leave the lower margin closely applied to the wax and the operation should be carried out by drawing a scalpel round the culture after it has been transferred to the disk for drying. The thin margin of the agar dries rapidly and gives the culture a good hold. The second method is to examine the drying culture five or six hours after its transfer to the disk and, if any peeling off has occurred, to press the extreme edge of the agar gently but firmly down. In this way it is usually possible to make it adhere again to the wax. These methods apply particularly to old cultures, as cultures not more than fourteen days old usually dry quite satisfactorily without either trimming or pressing and should, therefore, be used whenever possible.

The second difficulty was occasioned by the slowness of the drying due to coldness and dampness of the atmosphere. In such circumstances, it was found advisable to use some form of heat, and this practice, which has since proved advantageous for regular routine, is

recommended where any difficulty in drying is experienced. The heat derived from two 10-watt electric light bulbs connected in series has been found quite sufficient, drying the cultures within twenty-four hours, frequently less. This gives a temperature of approximately 23° C. to an inverted culture placed about 7 cm. directly above the lamp. The only danger is that the wax may become too soft in hot weather; a slightly plastic wax enables the drying culture to take a firm grip of it, but too soft a wax allows the drying agar to draw it up into wrinkles. Temperature conditions can be regulated by raising or lowering the drying culture and little difficulty has been found in making the adjustment. With care, hot-water radiators can be utilised as a source of heat.

Thirdly, instead of transferring the cultures by hand from the Petri dish to the drying disk it is sometimes a good alternative to float them out in water in a photographic dish. When the edge of the agar culture has been loosened, the Petri dish is held vertically and slowly lowered a little way into the water, the edge of the agar being raised by a lifter and induced to float on the surface. The Petri dish is gradually lowered more deeply into the water, at the same time very slowly rotated, preferably counter-clockwise, and gradually inclined to the horizontal, while the agar is separated from the glass by the lifter. Great care must be taken to prevent the water from flowing over the surface of the culture, which is somewhat difficult when the aerial mycelium is only poorly developed. By this procedure it is possible to loosen a culture which may have adhered to the glass by appressoria, and however fragile the agar may be, it can usually be transferred without being broken. Finally, the Petri dish is completely submerged and the agar culture floats free on the surface of the water. It can be gently guided over the drying disk already placed in the water in the photographic dish and the disk raised, leaving the agar culture lying flat on its surface. By adopting this method any kind of medium may be used for the culture; potato dextrose agar and Dox, as well as clear maize agar have consistently given satisfactory results.

Lastly, the black film of the ferrotype plate is liable to become detached round the cut edge in course of prolonged use and a stove-enamelled disk, with sloping edges and enamelled after being cut, has proved more efficient. The wax solution previously recommended must always be used in cleansing it. The disk may be mounted in the centre of a wooden tray* into which the wax can be melted. The use of such a tray has been found convenient, as the wax can be renewed without difficulty and the surface readily remade by flaming with a Bunsen.

* The drying disk with tray can be obtained from Messrs. Baird and Tatlock (London), Ltd., 14–17 Cross Street, Hatton Garden, London, E.C. 1.